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# SOME PROBLEMS ON CORNEAL PULSE WAVE (CORNEAL TRANSMITTED PULSE WAVE) OF THE RABBIT

Misao UYEMURA and Kikuo KAWASHIMA
Department of Ophthalmology, School of Medicine, Keio University

## Introduction

Studies on the diseases of the circulatory system, especially in case of hypertension and in its pathogeny have become popular; for instance, experimental methods of artificial hypertension and of encephalopathy etc.

The corneal pulse wave is a kind of wave motion transmitted directly from the pulsation of the central retinal artery, and is significant for the diagnosis in general circulatory system. Observation is done on rabbit eyeballs, because it is very difficult to record and recognize their corneal pulse wave, although they are similar in diameter to the human eyes.

The aim of this experiment is to examine in detail the conventional technique we use day by day. Some important factors which is arranged by the observation, are made clear during the experiment, and are to be reported in the present paper.

*Improved apparatus for experiment.* Keio University's electrical retinal artery sphygmotonometer with pick-up holder, (the latest type).—It'll be introduced in another paper.

*Animals for experiment.* Matured white rabbits, about 2kg. in weight.

## Experiment

1) *E.C.G.* has surely no direct connection with the blood circulation because of its muscular action current depending on heart muscular movement. But clinically E.C.G. can be regarded as the standard of pulse numbers. Now, E.C.G. of the rabbit is recorded. R-R interval of most rabbits' E.C.G. was 0.2 to 0.4 sec., 180 to 240/min. converting to pulse rate. Therefore, if the corneal pulse waves are caused by pulsation of the circulatory system, these must coincide with a period of E.C.G. The fact is namely that the corneal pulse wave has a 3-4 C.P.S. period.

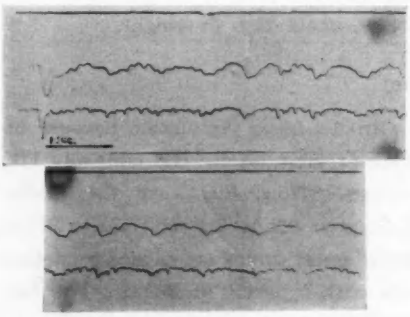
2) *Arrangement for filter.* The filter had to be selected for elimination of needless oscillation, and they were very useful for recording in similar figures and repetition. The vibration caused by hand-holding of the pick-up head differs according to the weight of the pick-up head and by construction of it. For its weight, the vibration is smallest at the weight of 220g. (experimenter's mean value).

If the weight is larger than 220g. the vibration is enlarged. Hypersensitive pick-up, therefore, was not suitable to the recording of the pulse waves for the sake mixing the ill effects of examiner's hand tremor. The frequency of vibration caused by hand tremor is almost ca 8 C.P.S. and the frequency of vibration peculiar to pick-up head was

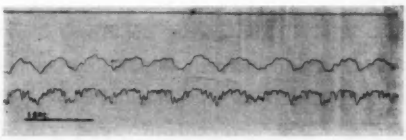
13 cycle wave. So 3-6 C.P.S. passing filter is necessary for accurate registering of a ca 4 C.P.S. chief wave element.

3) *Consideration for friction and weight of the pick-up head acting as receiver of corneal pulse waves.* At the recording of the corneal pulse wave, putting up the pick-up head resistance against the outside pressure, low cycle waves appeared synchronized with the rate of respiration (as shown in Fig. 1a). If breath-stress is given at the respiration in the way of recording of the corneal pulse wave, the wave changes the form together with the respiration change, then presently it comes back again (Fig. 1b). This fact suggested, that in this wave form series, the corneal pulse wave is not recorded and the wave of respiration which has a large wave amplitude is recorded in round form. In excitement, or in summer, the rabbit's respiration number becomes frequent and heavy, then the subjective corneal pulse wave is covered completely by respiration and can not be difficult to comprehend. Then the weight of pick-up is increased and the corneal pulse wave is recorded, and its wave form is shown as Fig. 2 a,b i.e., when the mass is light, the ground line in not removed, and if the ground line moves, the line soon returns as before. And when the mass is great, a 3-4 C.P.S. corneal pulse wave coinciding with E.C.G. period is hardly registered the pulse amplitude is narrower, and the low cycle wave of respiration appeared remarkably.

Fig. 1. (a), (b) Respiration's wave of rabbit

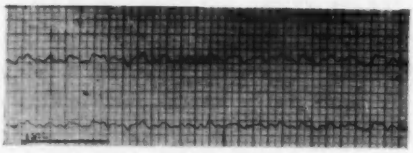


(a) Upperline: Pressure curve. Middle line: Respiration's wave by using a 2-1 filter. Lowerline: The same one by using a 1-10 filter

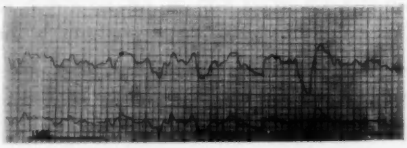


(b) Respiration's wave being changed by breath-stress

Fig. 2. (a), (b) Corneal pulse wave being influenced by pick-up head weight



(a) Recording by light pick-up head



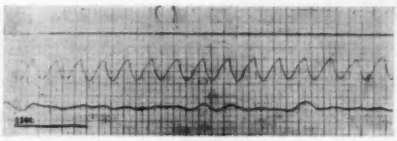
(b) Recording by heavy pick-up head

4) *Corneal pulse waves being influenced by ocular antero-posterior movement.* In a previous experiment it is found that at the time of corneal pulse wave recording, the wave of respiration is sometimes recorded by the use of some recording method. So the influence caused by the retrobulbar vessels was discussed. At the first step, the pressure sensing means were made lightly and the filter was put in.

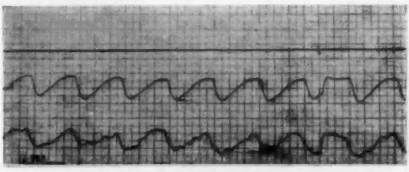
In this way, the corneal pulse wave was impossibly influenced by respiration. Secondly, the instrument was improved to find out the relative position between the pressure sensing means and pressure means. The record is shown as Fig. 3 a,b,c. In the rabbit's corneal pulse wave, the pulse wave picked up by pressure sensing means is recorded largely, but the changing curve of relative position influenced by ocular antero-posterior movement (by influence of the retrobulbar vessels and respiration) was very little.

But in the case of a human being, the pulse wave form considered to be caused by the retrobulbar vessels is clearly recorded even in a lower pressure.

Fig. 3. Corneal pulse wave and ocular antero-posterior movement wave.

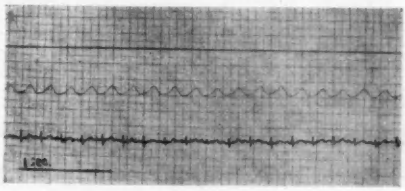


(a) In rabbit: Upperline: Pressure curve. Middle line: Corneal pulse wave. Lowerline: Ocular antero-posterior movement wave.

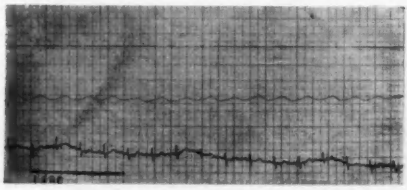


(b) In human beings

Fig. 4. (a), (b)



(a) Recording in side-down position (on lying down)
Upperline: Pressure curve
Middle line: Corneal pulse wave
Lowerline: E.C.G.



(b) Recording in natural posture

5) *About the rabbit's posture at the time of corueal pulse wave recording.*
The corneal pulse waves were recorded under the next two conditions ; the first is in side-down position (lying down), the second is in natural posture. Corneal pulse waves in both two conditions were compaired. As a result, it has been found that the first is higher recorded than the second (Fig. 4a, b).

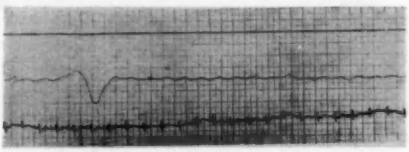
6) *Synchronous recording of corneal pulse waves relating to E.C.G. of normal rabbits.* The corneal pulse waves were recorded synchronously relating to E.C.G., as shown in Fig. 5, utilizing a filter which eliminates 4 to 10 cycle wave. The influences by retrobulbar vessels are very little in the rabbit's eyes, so the fixing point of the pick-up acting as the receiver for oscillation, is possible to settle at ground.

7) *Consideration for corneal pulse waves before and after cauterization of the central retinal artery.* Production of the corneal pulse wave could be experienced after cauterization of the central retinal artery along with the optic nerve, as shown in Fig. 6, results were just the same as in the former report. But the electronic cauterization can be the stimulus for nerves. Then we make an additional remark that the corneal pulse waves are influenced by cauterization.

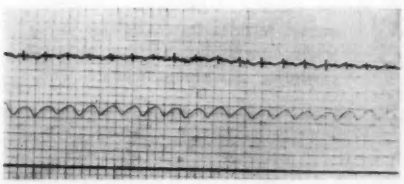
Fig. 5. Synchronous recording of a corneal pulse wave and E.C.G..

Upperline: Pressure ourve, Middle line: Corneal pulse wave, Lowerline: E.C.G.

Fig. 6 (a), (b)



(a) Corneal pulse wave before cauterization of central retinal artery. Upperline: Pressure curve. Middle line: Corneal pulse wave. Lower line: E.C.G.



(b) after cauterization

## Discussion

The study on the recording of rabbit's corneal pulse waves would be of great significance for research upon the diseases in the circulatory system. Distribution of energy upon the corneal pulse waves is very complicated, because the rabbit's eye is of half the size of the human one's and the arterial diameter is also smaller than human one's, but the lens is comparatively large.

Then the corneal pulse waves should be recorded under the following attention.

1) The corneal pulse wave should be recorded synchronously to E.C.G. for certification to their origin in the circulatory system. Because in recording of the rabbit's corneal pulse wave, we must step up the gains comparatively more than by recording human one's, so that the external oscillations are easily bursted into the waves.

(2) Various filters should be used for keeping up the waves in repetition and similarity, and a pick-up holder is available for more rigorous recording to be eliminated from ill effects.

But at this time, the eyeball is pressed from the upper side by a certain amount of pressure, and in addition the eyeball is pressed upperward from below by blood pressure of retrobulbar vessels, then the eyeball changes the form, and so its transforms act upon the pick-up as the corneal vibration. Such occured vibrations put upon the right corneal wave. This figure is shown as Fig. 3. Previously, in human eyes, the oscillation wave form, which is supposed to occur from the circulatory system, appears, but ordinarily, influences by the circulatory system appear slightly.

These respiration changes can be eliminated through the utilizing of various filters, and diminishing of inertial resistance and weight of pick-up head.

## Summary

The recording of rabbit's corneal pulse waves performs a significant part in experimental research on the cerebral vascular system, hence many factors upon the recording of corneal pulse waves were experienced, which can be summarized as follows.

1. Rabbit's corneal pulse waves were almost 3-4 C.P.S. waves, 180 to 248/min. in pulse rate.
2. Respiratory waves remarkably appeared with the increase of inertial resistance in the pick-up head.
3. A little ill effects due to ocular antero-posterior movement were caused by the circulatory system.
4. Corneal pulse waves appeared more largely on lying down than in a normal posture.
5. The synchronous recording to E.C.G. was fitted up with understanding of the corneal pulse waves.
6. On the basis of the above mentioned findings, the authors are sure, that the greater component upon the rabbit's corneal pulse waves depends on the central retinal artery.

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# STUDIES ON COLOR SENSATION AND CLINICAL USE

Kakichi UMAZUME

Department of Ophthalmology, Tokyo Medical College

The author and his co-workers have undertaken investigations on the color sensation during the last 18 years, and the present report summarizes the various aspects of these studies, which were presented at the 60th Annual Meeting of the Japan Ophthalmological Society, 1956[1),2)].

## I Illumination and Color sense

Color luminosity by means of orthochromatic photography is investigated by allowing the sensitivity curve of panchromatic plates to coincide with the luminosity curve of the eye under a certain condition.

*Changes of color luminosity under various conditions of sunlight*[3),4)] have been studied, since it has been shown that the spectral energy of the light-source varies according to the seasons of the year : the direction from which it comes, such as from north or south side : the weather conditions : the time of the day. A relatively constant luminosity is maintained throughout the year if the light-source is from the north side in clear weather.

*Artificial illumination*[5)]. CIE source C gives artificial light-source which is nearest to the average sunlight, but the apparatus is complicated and not commonly used. After investigating various types of artificial illumination, it has been found that the daylight fluorescent lamp approximates most closely the source C., and good results are obtained by using color vision test charts under this type of illumination.

## II Effects of contrast between the print and the background on reading and visual fatigue[6)]

Experimental findings indicated that one half of the subjects tested gave satisfactory responses with sharp contrast, such as black letters on a white paper, while the other half did the same when the contrast was less sharp, such as gray letters on a white paper,

## III Color harmony[7)]

On critically examining the theory of color harmony propounded by P.Moon and D.Spencer on experimental basis, it has been possible to confirm the greater part of their description on the relationship among the hue, value and chroma. However, the fact that there is an intermediate type must be noted. As to the aesthetic measure, we obtained a result different from theirs.

## IV New color vision tests

*Colorpyrometer (Mazda) test*[8)]. Inasmuch as this apparatus is based on the some principle as the anomaloscope (type 1), we used it with good results for the detection of color deficiency.

*Color discrimination test*(1)[9]. Letting the subject arrange 20 color patches on each of the Pitt's confusion lines of the protan and deutan (Fig. 1), the degree of his color deficiency was judged from errors committed in his arrangement. The results showed a significant relationship with those obtained by the anomaloscope test.

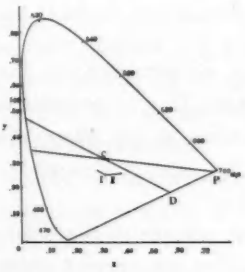
*Color discrimination test*(2)[10]. In conformity with Farnsworth-Munsell 100-hue test, we conducted color arrangement tests with all the 80 hues. Results showed that more errors had been made in yellow and between red-purple and purple in case of protan: and in yellow and between blue and blue purple in case of deutan. It is possible to tell protan from deutan by judging from the direction of errors on the side of cold colors. This can be explained by the cross angle by Pitt's confusion lines.

## V Environment and color sensation

1) Influences of the sound[11]

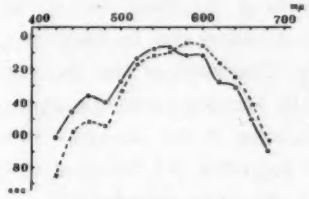
The sound (10db, 1000~) exerts influences both primary and secondary on the threshold in dark adaptation. By graphic presentation of the results the curves of stimulated and non-stimulated sensivity are found to cross each other at between 560m$\mu$ and 580m$\mu$: the spectral sensitivity stimulated by sound to ascend on the short wave side and to descend on the long wave side. A marked threshold fluctation is noted on the blue at 460m$\mu$–480m$\mu$. (Fig. 2)

Fig. 1




(Umazume)
Chromaticity of color patches.
P……Pitt's confusion line (Protan)
D……Pitt's confusion line (Deutan)
I……Color patches on protan's confusion line.
II……Color patches on deutan's confusion line.

Fig. 2




(Umazume)
Spectral sensitivity of the primary dark adaptation
×……×—Sound stimulation off.
○……○—Sound stimulation on.

2) Spectral sensitivity of the central fovea in primary dark adaptation[12]

*The influence effected by light adaptation.* The method by which sensitivity of the retina is determined consists in first finding the time required to recognize the subject. The maximum point of spectral sensitivity was found at 440–460m$\mu$, 540–560m$\mu$ and 600m$\mu$. The effects of treating the eyes with varying intensities of light adaptation on

the sensitivity of different chromatic light are compared, it was found that the greater the intensity, the lesser would be the sensitivity of the eye to the lights.

*The influence of chromatic pre-adaptation.* In general, spectral sensitivity towards chromatic light is found to be depressed when preadaptation measure is instituted. An increase appears to be most outstanding with red, particularly towards the shorter side of the wave length falling below 600m$\mu$. Likewise, preadaptation for orange leads to results in agreement with Wald's "violet receptor" showing an increase on the shorter side falling below 500m$\mu$. However, when preadaptation is applied for short wave length light, no increase in sensitivity is recognized on the corresponding longer side of the wave-length.

3) Influences of the motion.

Running and cold-water bathing make color sensation move towards the short-wave side of the spectrum.

## VI Acquired color deficiency

1) Influence of age on color sensation[13]

Experimental studies by the use of a colorpyrometer revealed the fact that the transparent media of the eye become discolored with advancing age, making the color sensation shift toward the long wave side of the spectrum, expanding at the same time the width of the Rayleigh's equation.

2) Changes due to diseases

a) The width of the Rayleigh's equation increases or decreases in the retinitis centralis Masuda according to the progress of the disease. This is probably due to the discoloration of the exudate. In testing color discrimination in the same disease, the result suggested the presence of congenital blue yellow blindess (tritanopia)[14].

b) Hepatitis epidemica[15]

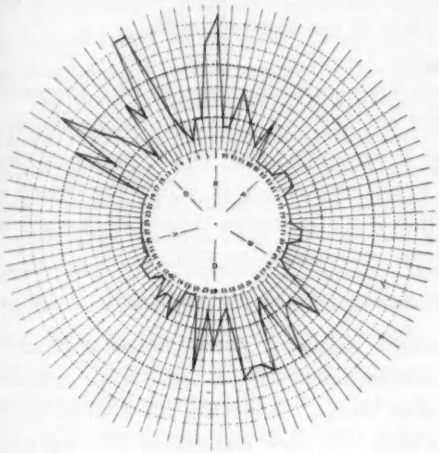
Icteric pigmentation in epidemic hepatitis causes the pointer of the colorpyrometer to slide toward the long wave side, although it returns to the normal position as the patient recovers.

c) Degeneratio pigmentosa retinae[16]

*The color vision in degeneratio pigmentosa retinae.* The color vision was examined by the F.-M.'s 100 hue test as well as by anomaloscope, the degrees being distinguished into the following 3 groups according to the process: *The first group* exhibits errors equal in order to those of normal vision, the Rayleigh's equation being unchanged. *In the second group* all hues confused but specially the confusion is marked from red to red-yellow and green to blue, the Rayleigh's equation being shifted to the side of red-yellow (Fig. 3). *The third group* shows a plain confusion in all hues, the Rayleigh's equation being not only shifted to the red-yellow, but also to the green-yellow side (Fig. 4). The results of the F.-M.'s 100 hue test on the CIE chromaticity diagram from the second

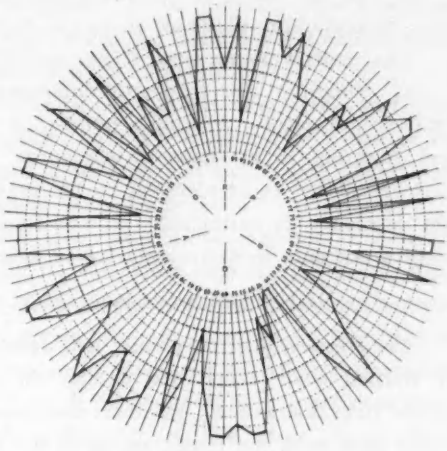
group are shown that the group is similar to the tritanope, namely, acquired blue-yellow blindness.

Fig. 3.



(Umazume)
Degeneratio pigmentosa retinae, second group.
Confusion color by F.-M. 100 hue test.

Fig. 4.



(Umazume)
Degeneratio pigmentosa retinae, third group.
Confusion color by F.-M. 100 hue test.

*The relationship between the normal vision on the central small field and acquired blue-yellow color blindness.* The color qualities of 63 color papers (1.0cm in diameter) made by combination of 9 warm and 7 cold colors on the CIE chromaticity diagram are examined in the normal vision and degeneratio pigmentosa retinae at distances varying from 0.5-3.0 meters.

The results are as follows:

1. Normal color vision on the central small field is similar to that of the acquired tritanope.

2. In the second group of this disease, the hues are greatly confused at shorter distances as compared with the normal. Moreover, in the first group of the disease, the hues are confused more markedly than normal vision at shorter distances.

3. The confusion loci on the CIE diagram are aggregated at the end of the shorter spectrum locus.

3) Central scotometer

Based on confusion of the hues in acquired blue-yellow color blindness a central scotometer was constructed, with which good results were obtained.

4) Color vision in acquired color anomalies under low luminance

Twelve kinds of luminance scale are placed in front of the CIE source C, and examinations with F.-M.'s 100 hue test are performed. Red, orange and blue-green are quickly confused in low luminance. Hue discrimination of yellow and purple is

still retained in low luminance.

VII Congenital color deficiency

1) On the basis of the CIE specifications, the color confusion of the tritans has been theoretically calculated to be due to the loss of the third element.

The neutral points were 477m$\mu$ and 578m$\mu$[17].

2) Sensitivity for chromatic differences on the neutral confusion locus in congenital red-green defects[18]. Placing a number of color patches on the neutral confusion lines, and selecting 18 of these as standards, the subject is allowed to choose the patches that are judged to be identical by his eyes. From the number of errors the degree of his deficiency is estimated. It was found that Pitt's confusion color theory could be applied not only to dichromats but to anomalous trichromats as well.

VIII The b-wave potential of the human ERG in the spectrum

*The b-potential of the normal and defective color visions to the monochromatic stimuli*[19]. In normal color vision and deutan the maximum magnitude of the b-wave indicated 510m$\mu$ light adaptation. Another maximum is at 560m$\mu$. In protan the distribution of single peak with the maximum at 500m$\mu$ was seen. In dark adaptation, all types have their maxima at 500m$\mu$. Purkinje's shift was recognized in the normals. Considering above the facts the peak at 560m$\mu$ appeared to be the photopic vision with cones, and that of 490 or 500m$\mu$ to be the scotopic with rods.

*The influence of chromatic preadaptation*[20]. After using the Mazda color filter for preadaptation, the ERG was examined in primary dark adaptation. In the preadaptation of red, orange and green, the peaks of the spectral sensitivity are at 500m$\mu$, but in blue, it removed to 520m$\mu$. The spectral sensitivity is differentially reduced by the chromatic preadaptation.

IX Pseudoisochromatic plates[20),21),22)]

1) Examining the plates of Ishihara, Oguchi, Ōkuma, Stilling and HRR in connection with Pitt's confusion lines on the CIE diagram and screeeing percentages, a critical evaluation as to the merits and demerits of each has been attempted. The screening efficiency of these plates, when the colors were placed on a confusion line, is high. On the other hand, that of the remaining plates, when the colors were placed on different confusion lines, is low.

2) The effect of illuminant A on color test plates[23)]

*CIE chromaticity changes of Ishihara test plates* are calculated under illuminant A. The degree of change is measured by means of Pitt's confusion loci. 73 color deficient individuals are examined under the same condition. To the protanopes, the chromaticity of the screening plates, having a tendency to be shifted in such a way as to coincide with the protanopic line, becomes illegible, which to the deuteranopes, for the same reason, becomes more readable. The qualitative plates become more readable to the

deuteranopes because of the fact that chromaticity of red-purple of these plates shift into the protanopic line. Under such conditions the protanopes may be readily mistaken for a deuteranopes.

*Similar investigations are made on Ōkuma's test plates.* When protanopes are examined by Okuma's test plates under illuminant A, they would be regarded as categorically unclassifiable, the deuteranopes to be qualitatively mild color blind.

*The effect of illuminant A is observed with Oguchi's test plates.* Due to the fact that the chromaticity of color disc of screening plates having a tendency to be shifted on to the protanopic line with low color saturation in the disc and shift being slight, the plates become unreadable to both protanopes and deuteranopes.

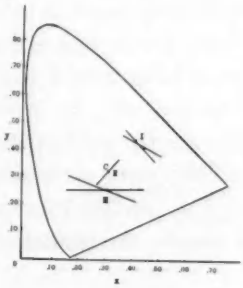
*The effect of illuminant A is observed in tests made with Stilling's plates.* The plates 3 and 4, with the chromaticity having a tendency of coinciding with the protanopic line, are illegible by protanopes and deuteranopes alike. The confusion effect is exerted upon protanopes and deuteranopes alike by plates 8,10 and 20 under A illuminant in spite of chromaticity abberations because of the fact that these plates are composed of many colors. In classification by the use of 5 to 20 plates, there is a great possibility that protanopes may be erroneously diagnosed as deuteranopes. A large chromaticity shift that may be shown on CIE diagram is due to the reaction of red-purple of the qualitative plates.

X New color vision test plates (Tokyo Medical College Color Vision Test)[24),25),26),27)]

We completed a new color vision test plate based on the theory of color science. These plates consist of a screening plate, a qualitative plate and a quantitative plate, including also a diagnostic plate for tritanopia.

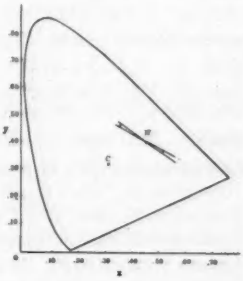
The chromaticity of these plates are shown in Fig. 5-6.

Fig. 5



(Umazume)
Chromaticity of plates (A)
I·····screening plate of protan and deutan
I·····screening plate of tritan
II·····qualitative plate

Fig. 6



(Umazume)
Chromaticity of plates (B)
IV·····quantitative plate
Lines show Pitt's confusion foci.

The accuracy of the screeing plate is as follows:

| | |
|---|---|
| Protan | 90.9% |
| Deutan | 97.4% |

The results obtained by the use of the qualitative plate and those of the anomaloscope agree to the extent of 96.6%.

By means of the quantitative plate, the protan and deutan are each divided into 3 classes according to their grades. Color blindness belongs to class 3.

The classification data by means of the quantitative plate were compared with the results given by the anomaloscope, but an agreement could not always be found between them. However, the results with a greater practicability will be obtained in this case than when a spectrum color testing method, such as the anomaloscope is used.

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(Other literatures are seen in each original articles)

# ELECTRON MICROSCOPIC STUDY OF ULTRATHIN SECTIONS OF SYMPATHETIC OPHTHALMIA (PRELIMINARY REPORT)

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In 1951 from the pathological study of 100 cases of sympathetic ophthalmia, a new theory for the pathogenesis of this disease was suggested by one of us (H.I.). It was proposed that a virus which has an affinity to chromatophores—"chromatotropic virus"—might be the pathogenic agent of this disease. The reason for this conclusion was arrived by the following pathological findings. 1. The essential pathological changes in the ocular tissues of sympathetic ophthalmia consist of the proliferation and the infiltration of leucocytes surrounding them. 2. With successive proliferation, the chromatophores take the form of so-called epithelioid cells. The pigment granules in these cells are not the ones phagocytosed. 3. The tissue reaction seems to be limited to the tissues containing the chromatophores. 4. The basophilic inclusion bodies of various sizes are occasionally found in the cytoplasm of the epithelioid cells. 5. In premature alopecia or in graying of the hair, and in the erythematous stage prior to vitiligo-like depigmentation of the skin, an inflammation is found around the hair roots and at the site where the dermal chromatophores exist. These inflammatory changes appear identical to those observed in the uveal tissues.

Following this pathological study, evidence for the existence of the virus was obtained by the study with intraocular materials (emulsions of uveal tissues or intraocular fluids) from the patients with this disease. When a filtrate of the materials through Seitz EK filter was injected into the rabbit eyes, severe inflammation resulted after a certain latent period. However the isolation of pathogenic agent through mice brain or chorio-allantoic membrane of developping chicken embryos had not been successful.

The purpose of this study is to confirm the authors' viral theory of sympathetic ophthalmia with electron microscope.

*Patient.* 23 year old man. A typical case of sympathetic ophthalmia occured after evisceration of his left eye injured by glass fragments of his spectacle. Severe uveitis of his right eye (sympathizing eye), fever, tinnitus, pleocytosis of cerebrospinal fluid, but no epilation of hair or no depigmentation of skin at that time.

The exciting eye (the atrophied eye after evisceration) was enucleated on October 18, 1956.

*Gross anatomy.* The enucleated eye was halved by knife. A large amount of uveal tissues remained was found between the sclera.

*Histological findings.* The typical nodules of epithelioid cells are found between the folds of the sclera, being surrounded by plasma cells and lymphocytes. The cytoplasm of epithelioid cells is acidophilic, and the nuclei of epithelioid cells are often becoming necrotic.

No giant cell was observed. In the cytoplasm of some epithelioid cells the strong basophilic globular inclusion bodies are found.

*Finding of smear preparation.* Smear preparations of uveal tissues were fixed with methanol and stained with Giemsa solution. Almost all of the epithelioid cells contain the pigment granules that are various in number and in size. The basophilic globular inclusion bodies are occasionally found in the cytoplasm of epithelioid cells that are becoming scanty in the pigment granules. They are 0.5 to 5$\mu$ in size. Some of them have a homogeneous internal structure, indicating a clear margin, but the others contain a few pigment granules. All of them show no tendency to be stainable acidophilic.

*Electron microscopic findings.* The pieces of specimen for electron microscopy were fixed in 1% buffered osmium tetroxide solution. After dehydration, they were embedded in a 8 : 2 or 7 : 3 mixture of n-butylmetacrylate and methylmetacrylate and sectioned with JUM 3 ultramicrotome of Japan Electron Optics Laboratory Company and Porter-Blum ultramicrotome. The electron microscope used was the one of JUM 4 C type of Japan Electron Optics Laboratory Company.

Cells other than the epithelioid cells did not show any outstanding changes, so the changes of the epithelioid cells are described here.

1. Cell membrance. The cell membrane of the epithelioid cells appear as a well defined line. When many cells were in close contact, the cell membranes insert each other, showing complicated folds and the extension of small prickles into the intercellular spaces.

2. Nucleus. The nucleus is bounded by a clear nuclear membrane which show often the double layers, being separated by a less opaque interspace. The nucleoplasm is composed of fine granular material. The nucleoli, 1 or 2 in number, are swollen and filled with homogeneous or granular mass. The nucleoli persist until the last stage of the epithelioid cell.

3. Cytoplasm. The cytoplasm of the epithelioid cells reveals the following characteristic changes.

a. Vacuolization. The epithelioid cells are markedly swollen due to the large number of vacuoles which are derived from enlarged endoplasmic reticulum sacks and enlarged hollow mitochondria.

b. Degeneration of mitochondria. In the epithelioid cell mitochondria increase in number and tend to be larger than normal. They exhibit very often the degenerative changes of various degree. The degenerating mitochondria become much larger than normal and show breaks in their limiting membrane. The double nature of their limiting membrane, however, can be clearly seen at some site around their circumference. The interiors of such mitochondria contain the remnants of cristae and the complicated membranes transformed from the cristae and a great deal of granular material, 10 to 20 m$\mu$ in diameter which are often arranged in irregular clumps.

These mitochondria seem to be in some way related to the formation of pigment on one hand and with proliferation of virus-like particles on the other.

c. Pigment formation. In the epithelioid cells various phases of melanin pigment formaticed are noticed. A lot of distinct pigment granules, various in size, gather in groups and are enclosed within the capsule. This finding seems to coincide to the "mature form of pigment formation center in melanoblast" (Weissenfels) and might be a normal process of pigment formation.

Besides this, various findings of pigment formation are noticed. Minute pigment granules are formed in the vacuoles apparently derived from endoplasmic reticulum sacks and seem to be increasing in size.

There are observed the ellipsoid bodies that contain the powder-like or almost homogeneous material of low electron density and those that contain the fine granular material of relatively high electron density with small pigment granules. Sometimes from within the ellipsoid bodies the pigment granules appear to be released into the cytoplasm. These fine electron-dense granules are thought to be promelanin granules. These findings observed in the ellipsoid bodies seem to be in the stage where pigment formation is not advanced, and might be considered as the "empty phase of pigment formation" (Weissenfels). However these finding of abnormal pigment formation are presumably the disturbance phase of pigment production, because the epithelioid cells are considered as the disturbance phase of normal chromatophores by some pathologic factor. At any rate it is shown that the epithelioid cells have the ability to form the pigment granules. So our opinion that the epithelioid cells are derived from chromatophores, are justified.

d. Inclusion bodies. It is not easy to observe the inclusion bodies under electron microscope because of relative rarity of their appearance in the epithelioid cells. There are great variations in form and in size of the inclusion bodies.

As a form of inclusion bodies, the ellipsoidal bodies, 3 to 8$\mu$ in size, surrounded by the incomplete capsule, are noticed. The internals of them consist of matrix-like substance of low electron density and the amorphous particles of various sizes that are scattered among the matrix and are of high electron density. These bodies look like the inclusion bodies in the bronchial epithelium of mice infected by inhalation of aerosols of influenza virus (Hartford et al, 1955). Because of their similarity with degenerating mitochondria, the formation of this of inclusion bodies may possibly be derived from the mitochondria.

Also observable are the inclusion bodies of another type. They contain the osmiophilic matrix of high electron density and the altered mitochondria around or in the matrix as well as the small pigment granules. The bodies are lined by the membrane that are sometimes not clear. At the present time it is not clear whether these types of inclusion bodies are of different kind or they represent the different stages of the same inclusion bodies.

e. Virus-like particles. The groups of particles are visible around or in some of the inclusion bodies and even in the cytoplasm close to the latter. The particles posses the characteristic internal structure consisting of a core (40 to 75 m$\mu$ in size) of low electron density enclosed by the opaque shell of high electron density about 25 m$\mu$ in thickness, giving an over-all diameter ranging from 65 to 100 m$\mu$. Some of the particles have a

Fig. 1. Considerable variation in the state of preservation of mitochondria in the epithelioid cell. ×21,000. m′: Degenerating mitochondria containing the cristae that are disorganized and becoming cystic. m″: Degenerating mitochondria containing the granular material considered as the remnants of cristae.

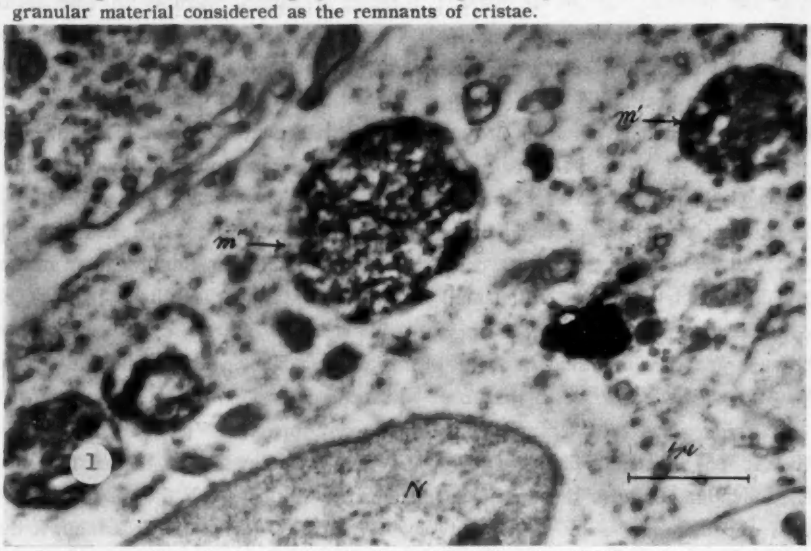



Fig. 2. Degeneration of mitochondria in the epithelioid cell. ×17,000. m′: Degenerating mitochondria surrounded by the incomplete double membrane. Cristae converted into granular mass. m″: Degenerating mitochondria. In its central part the granular material of high electron density is visible. The peripheral part of it is filled with the fine granular electron-lucent material.

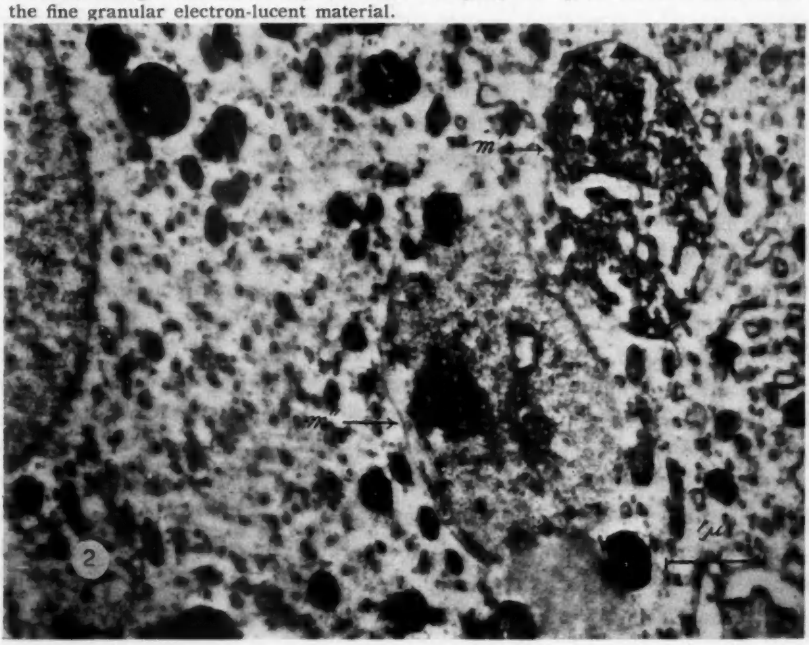


Fig. 3. Normal pigment formation in the epithelioid cell. ×17,000. P.E.C.: This coincides with the "mature form of pigment formation center in melanoblast" (Weissenfels).

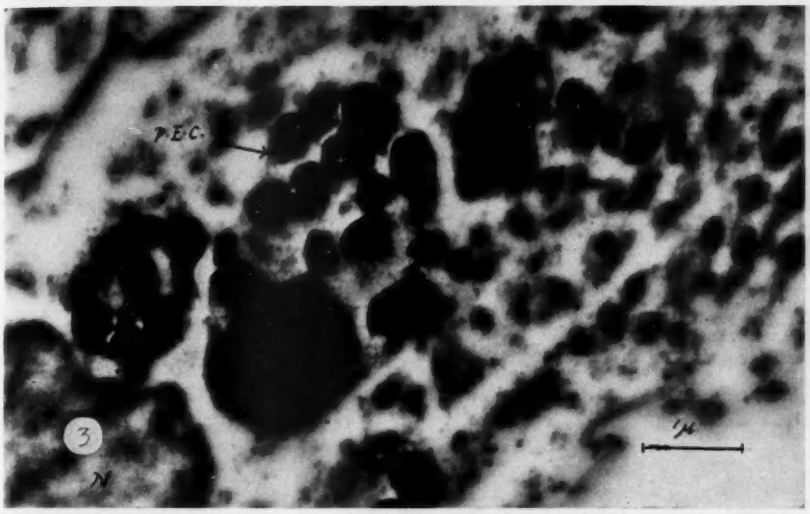



Fig. 4. Abnormal pigment formation in the epithelioid cell. ×32,000. p′: The ellipsoid body containing a few pigment granules and electron-dense granular mass considered as promelanin granules. P″: The ellipsoid body containing the fine granular electron-lucent mass and several distinct pigment granules.

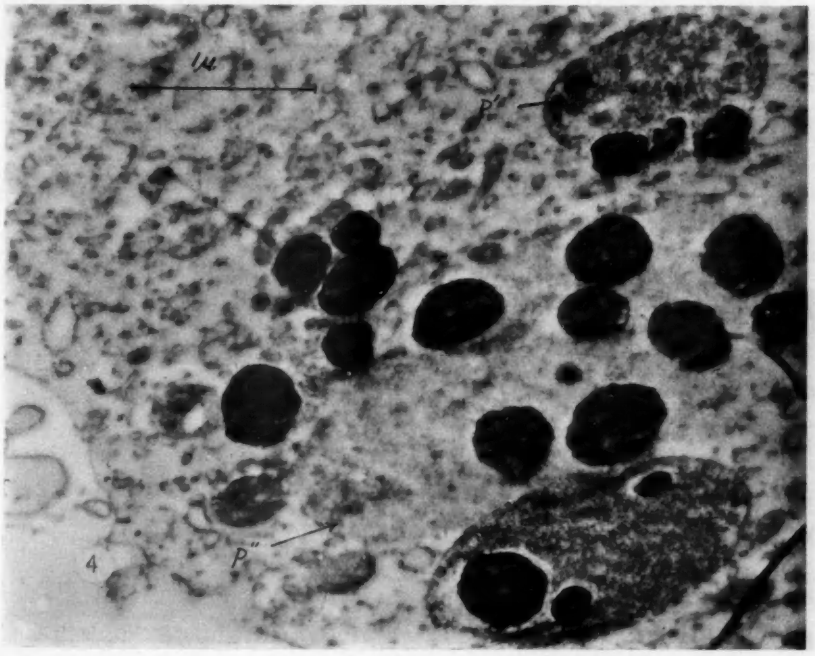


Fig. 5. Abnormal pigment formation in the epithelioid cell. ×32,000. P'': The ellipsoid body containing the fine granular electron-lucent mass surrounded by endoplasmic reticulum.

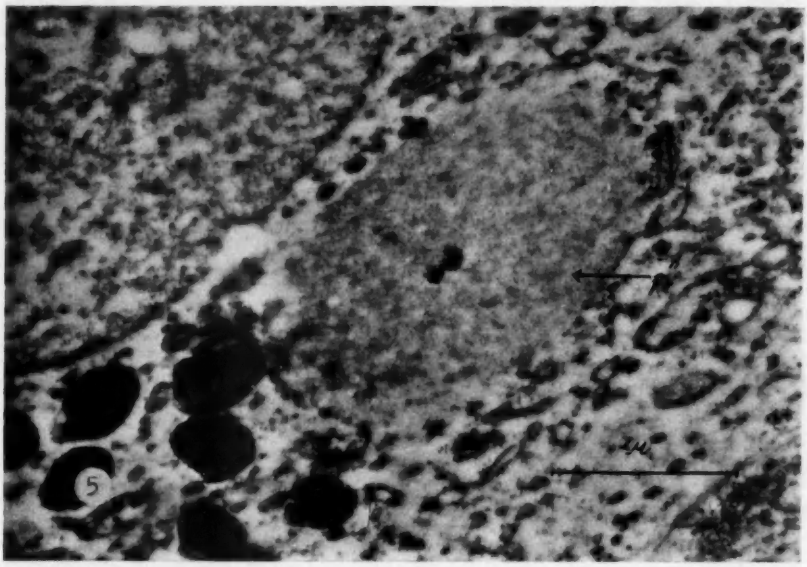


Fig. 6. Cytoplasmic inclusion body in the epithelioid cell. ×17,000. The inclusion body is surrounded by the incomplete membrane and contains a matrix of moderate electron density (a) and amorphous granules (b), and also hollow mitochondria (m) in its peripheral part.

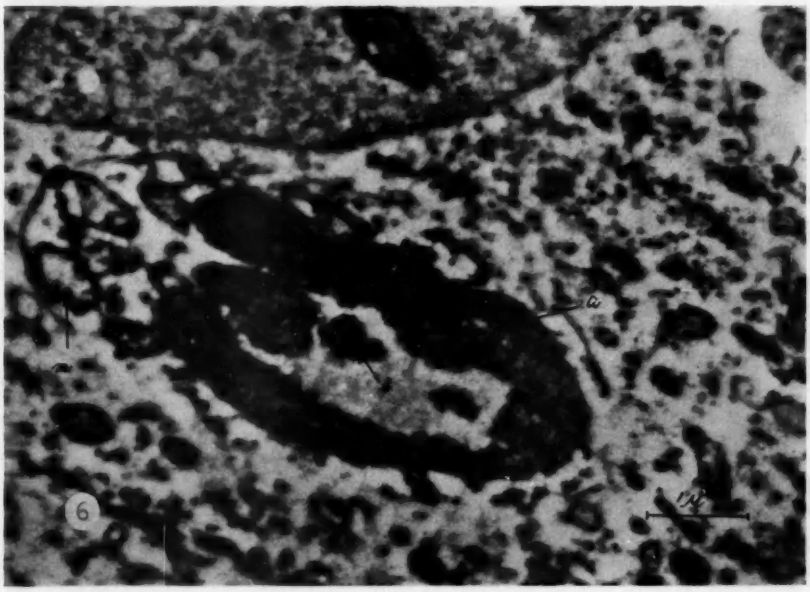


Fig. 7. Cytoplasmic inclusion body. ×31,000. It contains the amorphous matrix(Ma), the reticular mitochondria(Ev)and the modified endoplasmic sack(Ers). Around the inclusion body, or in the cytoplasm near the inclusion body opaque ring-shaped particles are visible. Some particles are embedded in the matrix.

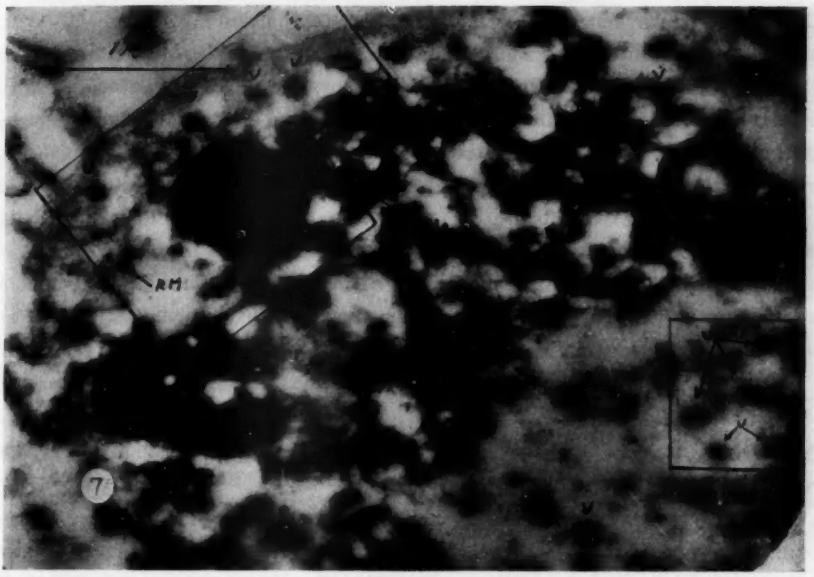
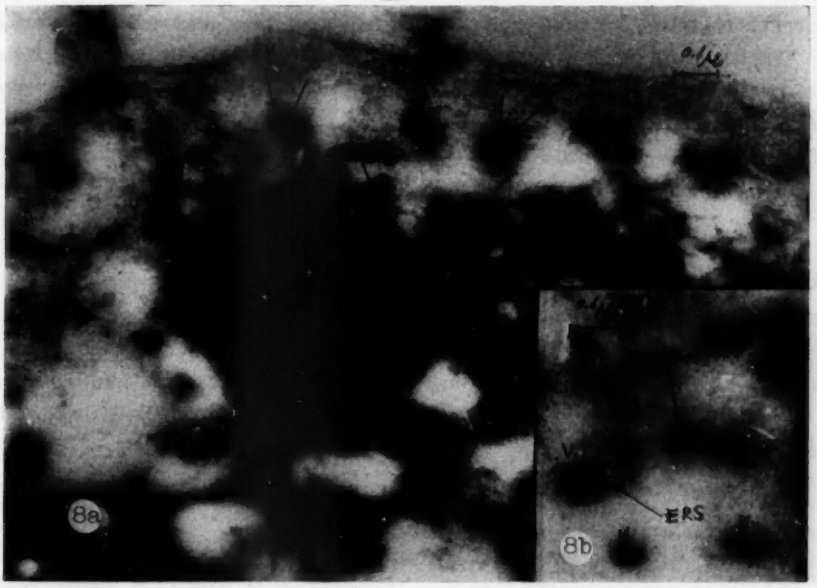



Fig. 8 Enlarged portion of Fig. 7. ×65,000. The transparent core(c) 40 to 75 mμ in diameter was surrounded by the shell(s) which has frequently close-fitting envelope(Ev).
Fig. 8b. Enlarged lower portion of Fig. 7. ×65.000. Opaque ring-shaped particles(v) can be seen in endoplasmic reticulum (Ev).



close-fitting envelope (endoplasmic reticulum ?) around the shell. Occasionally the particles of almost uniform size are found in the membrane that surrounds the inclusion. These particles are never recognizable in the normal chromatophores.

It is not possible to decide whether these particles identified in the epithelioid cells are actually identical with virus particles. However these particles are associated with some matrix substance and seem to have some relation with the apparently altered mitochondria, and appear in groups, showing little variation in size, and have a definite internal structure, showing an excessive electron density compared with other cytoplasmic particles. Furthermore they bear strong resemblance to the bodies of distinctive morphology that are believed to be virus particles of influenza by Morgan (1954), and also to the bodies that are considered as virus particles of Ehrlich ascites tumor by Friedlaender et al. (1956). These evidences suggest the possibility that these are the virus particles.

## Conclusion

The ultrathin sections of the uveal tissues of sympathetic ophthalmia were observed by electron microscope.

1. In the epithelioid cells in the tissues of sympathetic ophthalmia, the normal and abnormal processes of melanin pigment formation were noticed. This fact, together with the findings obtained by histological examinations, suggests that the epithelioid cells in this disease are derived from the chromatophores.

2. The basophilic inclusion bodies of various sizes are sometimes found in the cytoplasm of the epithelioid cells. The electron microscopic finding of the inclusion bodies is not simple.

3. The virus-like particles were found around or in some of the inclusion bodies. The possibility of exsistence of virus that had an affinity to chromatophores was proved, but further investigations are necessary.

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# CREATINE PHOSPHOKINASE IN RETINA

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## Introduction

Tawara described that CP in retina diminishes by illumination of light and Akiya confirmed it and also described that the close relation existed between the tissue respiration and CP. While, Ezuka reported that ATP diminishes by light illumination and Sugita also recognzied it experimentally in vitro.

It therefore seems that the high energy phosphate bond of ATP and CP is important in the function of retina. It is deduced that the role of this high energy phosphate bond is different from that of muscle contraction. In muscle contraction the high energy phosphate bond of ATP is immediately provided from CP by CPK. It seems that such a mechanism also exists in retina. From this point of view, we have researched the mechanism of the splitting of CP. Consequently we found that it occured according to Lohmann's Reaction and only ADP could function as an acceptor of CP by using a purified enzyme of retina, and some of the properties of CPK were also studied.

## Material and Method

*Substrate.* CP was prepared as Ca-salt by the method of Zeile & Fawaz. ATP-$Ba_2$ salt was separated from the muscle of a rabbit by the method of Kerr. ADP was prepared from ATP by the method of Lohmann & Schuster. AMP was of a commercial preparation obtained from Light & Co., Ltd.

*Buffer system.* 0.1M Glycylglycine, 0.1 M Histidine-NaOH, Glycine-NaOH were used.

*Analytical method.* Inorganic P and CP were measured by the method of Lohmann and ATP was determined as $\Delta$7P.

*Preparation of enzyme.* The retinae were removed from eyes of calf or rabbit, and homogenized with cold 0.9% NaCl solution using glass homogenizer. Then they were centrifuged and the supernatant was dialysed against cold running water for more than 24 hours. The dialysed retinal extract was used as an enzyme solution.

A purified enzyme was obtained by the method of Lardy. But the retinae were not obtained in a large quantity and so we stopped at the interim process. 15 pieces of calf retinae were removed and homogenized with 20 c.c. of cold $5\times10^{-3}$M KCl solution. When it was centrifuged its precipitant was again homogenized with 10 c.c. of KCl and recentrifuged. The supernatant thus obtained was collected and $NH_4Cl$ was added to the final concentration of 0.1M and pH was adjusted to 9.0 with dilute NaOH. The enzyme

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*Abbreviations used:*
*ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine-5-phosphate; CP, phosphocreatine; CPK, creatine phosphokinase: PCMB, p-chlormercuribenzoate; GSH, glutation; $\Delta$7P, liberated inorganic P in IN HCl, 100°C, 7min.*

solution was cooled at -5°C with dry-ice acetone and to it 1.5 vol. of cooled ethanol was added while it was being shaken severely. Then 2M $MgSO_4$ (pH8.5) was added to the final concentration of $3\times10^{-2}$M and shaken. After it was centrifuged the sediment was collected and suspended with cooled Histidine Buffer (pH 6.0). This was centrifuged again and the sediment was washed twice and suspended with dilute Histidine Buffer (pH. 7.0). Protein in the enzyme solution amounted to about 8%.

## Results

The splitting of CP was researched with the dialysed or undialysed retinal extract. Reaction mixture contains 5% retinal extract 1.0 c.c., 0.1M Glycylglycine (pH 7.0) 1.0 c.c. and 0.01M CP-Na 1.0 c.c.. As shown in Table 1, the splitting of CP occurs rapidly in the undialysed extract, but slightly in the dialysed extract. From this fact, it is apparent that the dialysable co-ferment would be necessary in the splitting of CP. If the splitting of CP in retina also occurs according to Lohmann's reaction, such a co-ferment must be AMP.

Table 1. The splitting of phosphocreatine by the dialysed or undialysed retinal extract
Incubation Temperature 37.5°C

| reaction time(min.) | liberated inorganic P γ/0.5 cc | |
|---|---|---|
| | undialysed | dialysed |
| 0 | 3.9 | 1.15 |
| 15 | 29.95 | 2.25 |
| 30 | 43.35 | 2.75 |
| 45 | 46.55 | 3.25 |
| 60 | 46.35 | 3.45 |

Table 2. The splitting of Phosphocreatine by the dialysed retinal extract with adding up AMP or none
Incubation Temperature 37.5°C

| | reaction time (min.) | P γ/0.5 cc | | |
|---|---|---|---|---|
| | | Inorganic P | Phospho-creatine P | ⊿P |
| AMP added | 0 | 1.7 | 31.9 | 0 |
| | 20 | 15.3 | 4.3 | 14.4 |
| | 40 | 20.08 | 4.12 | 7.8 |
| | 60 | 27.08 | 4.7 | 2.6 |
| none | 0 | 1.7 | 32.3 | 0 |
| | 20 | 1.9 | 31.3 | 0 |
| | 40 | 2.6 | 30.6 | 0 |
| | 60 | 2.1 | 31.5 | 0 |

So we added AMP to the dialysed extract as shown in Table 2. Reaction mixture contains dialysed extract 3.0 c.c., $10^{-3}$M CP 2.0c.c., $10^{-3}$M AMP 2.0 c.c. and 0.1M Glycylglycine (pH 7.0) 3.0 c.c.. In absence of AMP, CP scarcely splits, while in its presence CP rapidly splits and ATP is synthetized simultaneously. Namely, the splitting of CP in retina is caused by CPK activity and not by phosphoamidase.

Table 3. Reaction rate with time of the foward reaction
Incubation Temperature 37.5°C

| reaction time (min.) | P γ/0.5 cc | | |
|---|---|---|---|
| | Inorganic P | Phospho-creatine P | ⊿7P |
| 0 | 1.5 | 25.9 | 0 |
| 5 | 4.1 | 3.1 | 16.6 |
| 10 | 5.0 | 2.2 | 16.0 |
| 15 | 6.0 | 1.0 | 16.8 |
| 20 | 6.8 | 2.0 | 15.0 |
| 25 | 6.8 | 2.4 | 14.6 |

Table 4. PH optimum of the forward reaction
Incubation Temp. 37.5°C, Time 10 min.

| Buffer | pH | P γ/cc | | |
|---|---|---|---|---|
| | | Inorganic P | Phospho -creatine P | ⊿7P |
| Histidine | 5.0 | 2.6 | 30.2 | 1.4 |
| | 5.5 | 2.8 | 23.8 | 9.0 |
| | 6.0 | 3.0 | 14.0 | 14.7 |
| | 6.5 | 3.6 | 11.2 | 20.8 |
| | 6.8 | 4.0 | 9.0 | 24.2 |
| | 7.0 | 4.0 | 10.7 | 22.0 |
| | 7.5 | 3.8 | 17.6 | 15.6 |
| | 8.0 | 3.6 | 21.2 | 13.0. |
| Glycine | 8.5 | 3.4 | 27.4 | 7.0 |
| | 9.0 | 3.2 | 29.0 | 6.0 |
| | 10.0 | 2.2 | 38.0 | 0 |
| | 11.0 | 2.0 | 37.4 | 0 |
| O Time | 7.0 | 1.0 | 38.0 | 0 |

*Reaction rate* (*Table 3*). The rate of reaction progress was measured by 5% dialysed extract as in the previous experiment. It is clear by this experiment that the splitting of CP is completed in less than 5 min.

*PH optimum of forward reaction* (*Table 4*). The optimal pH was determined with the dialysed extract. As to buffer, Histidine NaOH (pH 5.0-8.0) and glycine-NaOH (pH 8.5-11.6) were used. Each reaction mixture contains dialysed extract 0.4 c.c., buffer 0.8 c.c., $10^{-2}$ M CP 0.4 c.c. and $10^{-2}$ AMP 0.4c.c.. The pH optimum for forward reaction of CPK lies at pH 6.8.

*The synthesis of phosphocreatine from ATP and Creatine* (*Table 5*). CPK in muscle and brain catalyses the CP synthesis from ATP and creatine. And then we investigated into the reverse reaction of CPK as follows. Reaction mixture contains dialysed extract 2.0 c.c., 0.1 M Glycine-NaOH (pH 8.9) 3.0 c.c., $5\times10^{-3}$M ATP 2.0 c.c., $5\times10^{-2}$M Creatine 1.0 c.c. & $5\times10^{-2}$ M NaF 2.0 c.c.. The inorganic P, CP and $\Delta$7P were measured at the time of 0′, 5′, 10′, 15′, 20′ and 25′. Consequently, 33.4$\gamma$ of ATP diminished to 21.8$\gamma$ after 5 minute-incubation and simultaneously 9.2$\gamma$ of CP was synthetized. NaF was added as the inhibitor of ATP-ase activity, but no effect was found by the addition of it. From this result it is confirmed that ATP is decomposed by CPK and CP is synthetized. This reverse reaction seems to occur rapidly within 5 min. but thereafter the reaction progress turns slower.

Table 5. The synthesis of Phosphocreatine from ATP and Creatine
Incubation Temp. 37.5°C

| | reaction time (min.) | P $\gamma$/0.5 cc | | |
|---|---|---|---|---|
| | | Inorganic P | Phospho-creatine P | $\Delta$7P |
| NaF added | 5 | 3.4 | 9.2 | 21.8 |
| | 10 | 3.9 | 12.7 | 17.8 |
| | 15 | 4.6 | 15.8 | 13.8 |
| | 20 | 5.4 | 15.9 | 13.2 |
| | 25 | 6.5 | 16.0 | 12.0 |
| none | 5 | 2.4 | 9.6 | 22.4 |
| | 10 | 3.6 | 12.2 | 18.6 |
| | 15 | 3.9 | 14.6 | 15.6 |
| | 20 | 6.0 | 14.8 | 13.8 |
| | 25 | 6.0 | 14.9 | 12.6 |
| O Time | 0 | 1.0 | 0 | 33.4 |

*PH optimum of CP synthesis.* The reaction mixture contains dialysed extract 0.4 c.c., buffer 1.0 c.c., $10^{-1}$ M Creatine 0.2 c.c. & $5\times10^{-3}$ M ATP 0.4 c.c.. As shown in Table 6 the pH optimum lies at pH 9.0.

Table 6. PH optimum of the reverse reaction
Incubation Temp. 37.5°C, Time 15 min.

| Buffer | pH | P γ/0.5 cc | | |
|---|---|---|---|---|
| | | Inorganic P | Phospho-creatine P | ⊿P |
| Histidine | 5.0 | 3.2 | 1.6 | 28.4 |
| | 6.0 | 3.6 | 2.4 | 28.0 |
| | 7.0 | 3.6 | 4.8 | 26.0 |
| | 7.5 | 4.0 | 5.6 | 21.0 |
| | 8.0 | 4.0 | 7.4 | 16.6 |
| Glycine | 8.5 | 5.8 | 12.6 | 14.0 |
| | 9.0 | 6.0 | 15.4 | 12.8 |
| | 9.3 | 5.8 | 14.5 | 14.0 |
| | 9.6 | 5.5 | 14.0 | 17.6 |
| | 10.0 | 4.1 | 12.3 | 24.4 |
| | 11.0 | 2.8 | 9.6 | 26.0 |
| | 12.0 | 2.2 | 5.2 | 28.0 |
| O Time | 9.0 | 2.2 | 0 | 30.0 |

*P-Acceptor of CPK activity.* In the previous experiment AMP seems to function as a P-acceptor of CPK activity, but the enzyme solution was inpurified, so in consideration of the view of Ennor & Rosenberg here arises a problem whether ADP or AMP could truly function as an acceptor. Narayanaswami described that ADP was incomplete and AMP was quite complete as P-acceptor for the CPK activity. On the other hand, Ennor & Rosenberg concluded that only ADP acted as the P-acceptor using the purified enzyme. Therefore researches were made into the CPK activity with adding up AMP or ADP in the reaction mixture. It contains dialysed extract 2.4 c.c., 0.1 M Histidine buffer (pH 6.8) 1.2 c.c., $10^{-2}$M CP 0.6 c.c., $10^{-2}$M AMP or ADP 0.6 c.c. & 0.1 M $MgCl_2$ 0.6 c.c.. As shown in Table 7, ADP seems to act as P-acceptor as well as AMP, but the dialysed extract may include Adenylate-kinase and other various enzyme besides the CPK. Therefore purified enzyme of CPK must be used for the determination of P-acceptor. The purified enzyme was obtained from the calf retina by the method of Lardy with slight modification. The activity of CPK was measured in the addition of AMP or ADP to the purified enzyme. The reaction mixture contains the purfied enzyme 0.6 c.c., $10^{-2}$ M CP 0.6 c.c., $10^{-2}$ M AMP or ADP 0.6 c.c., 0.1 M $MgCl_2$ 0.6 c.c. & 0.1 M Histidine buffer (pH 6.8) 3.0 c.c.. As shown in Table 8, in addition of AMP the splitting of CP was 0, but when ADP is added the spltting of CP occurs. So it was confirmed that only ADP could function as the acceptor of CPK.

Table 7. The splitting of phosphocreatine by the dialysed extract with adding up AMP or ADP
Incubation Temp. 37.5°C

| | time (min.) | P γ/0.5 cc | |
|---|---|---|---|
| | | Inorganic P | Phosphocreatine P |
| AMP added | 0 | 1.5 | 15.5 |
| | 10 | 6.2 | 1.8 |
| | 30 | 12.4 | 1.6 |
| ADP added | 0 | 2.0 | 15.5 |
| | 10 | 15.3 | 4.1 |
| | 30 | 23.2 | 4.0 |

Table 8. The splitting of phoshocreatine by the purified enzyme with adding up AMP or ADP
Incubation Temp. 37.5°C

| | time (min.) | P γ/0.5 cc | |
|---|---|---|---|
| | | Inorganic P | Phosphocreatine P |
| AMP added | 0 | 4.4 | 20.0 |
| | 20 | 4.0 | 21.0 |
| | 50 | 4.1 | 20.3 |
| ADP added | 0 | 4.0 | 21.0 |
| | 20 | 4.1 | 15.5 |
| | 50 | 5.5 | 13.7 |

*Effect of inorganic ion.* The effect of inorganic ion on the activity of CPK was studied. The reaction mixture contains 0.1 M Histidine buffer (pH 6.8) 1.0 c.c., purified enzyme 0.2 c.c., $10^{-2}$ M CP 0.2 c.c., $10^{-2}$ M ADP 0.4 c.c. & salt solution 0.2 c.c.. As shown in Table 9, CPK is activated by $Mg^{++}$, slightly by $Ca^{++}$, scarecely by $K^{+}$.

*The inhibitory effect of PCMB and the reversal of its inhibition by GSH.* It is considered that CPK belongs to the -SH enzyme. We investigated into the inhibitory effect of PCMB, which had been used in the characterization of -SH enzymes. The reaction mixture contains 0.1 M Histidine buffer (pH 6.8) 0.8 c.c., purified enzyme 0.2 c.c., $10^{-2}$ M CP 0.2 c.c.,$10^{-2}$ M ADP 0.4 c.c., PCMB 0.2 c.c. & GSH 0.2 c.c.. As shown in Table 10, the inhibitory effect is evident at the final concentration of $10^{-4}$ M PCMB. Considering that the inhibitory effect of PCMB is due to the fixation of -SH groups of the enzyme, it will be released by the addition of GSH. Therefore we investigated into the reversal effect of GSH on the PCMB inhibition. The reaction mixture contains 0.1 M Histidine buffer 0.8 c.c., purified enzyme 0.2 c.c., $10^{-2}$ M CP 0.2 c.c., $10^{-2}$ ADP 0.4 c.c., $2\times10^{-3}$ M PCMB 0.2 c.c. & GSH 0.2

c.c.. As shown in Table 11, the inhibitory effect of PCMB is completely released by GSH when GSH is added at the same final concentration as that of PCMB. Further, CPK is more activated by the addition of GSH than when there is no PCMB. From the results of Table 10 and Table 11 it is confirmed that CPK belongs to a typical -SH enzyme.

## Discussion

In the skeletal muscle the splitting of CP occurs in the presence of AMP and the phosphate bond of CP is transferred to AMP and consequently ATP is synthetized. Namely, while the splitting of CP takes place according to Lohmann R., Ichihara reported the activity of phosphoamidase in kidney which catalyses the following reaction.

Table 9. Effect of inorganic ion on the splitting of CP
Incubation Temp. 37.5°C, Time 50 min.

| Ion | final concentration (M.) | P γ/0.5 cc | |
|---|---|---|---|
| | | Inorganic P | Phosphocreatine P |
| $Mg^{++}$ | $10^{-2}$ | 4.7 | 11.9 |
| | $2\times10^{-3}$ | 4.4 | 16.2 |
| $Ca^{++}$ | $10^{-2}$ | 2.0 | 18.8 |
| | $2\times10^{-3}$ | 3.9 | 13.7 |
| $K^{+}$ | $10^{-2}$ | 4.2 | 15.4 |
| | $2\times10^{-3}$ | 4.9 | 14.7 |
| none | 0 | 3.6 | 16.4 |
| O Time | 0 | 3.2 | 19.6 |

Table 10. The inhibitory effect of P.C.M.B. on the splitting of CP
Incubation Temp. 37.5°C, Time 50 min.

| final concentration of P.C.M.B. (M.) | P γ/0.5 cc | |
|---|---|---|
| | Inorganic P | Phosphocreatine P |
| $2\times10^{-4}$ | 3.3 | 19.7 |
| $10^{-4}$ | 3.6 | 19.4 |
| $10^{-5}$ | 4.9 | 11.9 |
| $10^{-6}$ | 5.0 | 11.0 |
| $10^{-7}$ | 4.7 | 11.2 |
| 0 | 4.7 | 11.9 |
| O Time | 3.2 | 19.8 |

Table 11. The reversal of P.C.M.B. inhibition by GSH
Incubation Temp. 37.5°C, Time 50 min.

| final concentration (M.) | | P γ/0.5 cc | |
|---|---|---|---|
| P.C.M.B. | GSH | Inorganic P | Phospho-creatine P |
| $2\times10^{-4}$ | $10^{-3}$ | 4.4 | 11.6 |
| 〃 | $2\times10^{-4}$ | 4.3 | 11.7 |
| 〃 | $10^{-4}$ | 3.4 | 19.8 |
| 〃 | $10^{-5}$ | 3.6 | 19.7 |
| 〃 | 0 | 3.4 | 19.2 |
| 0 | 0 | 4.6 | 11.8 |
| O Time | 0 | 3.0 | 19.8 |

$$CP+H_2O \rightleftharpoons Creatine+H_3PO_4$$

It must be discussed that the splitting of CP in retina occurs whether by CPK or by phophoamidase. As we previously described, the splitting of CP occurs rapidly in the undialysed extract but scarcely in the dialysed. Nevertheless, when AMP added in the dialysed extract it occurs rapidly and ATP is synthetized simultaneously. Consequently, it may be recongnized that the splitting of CP occurs only by CPK activity and the phosphate bond of CP is transferred to the Adenylic-acid system. The activity of phosphoamidase is almost negligible.

Lohmann (1934) explained the mechanism of the splitting of CP by the following formula : -

$$ATP \rightleftharpoons AMP+2 \text{ ortho-phosphate}$$
$$AMP+2CP \rightleftharpoons ATP+2\cdot Creatine$$

while, Lehmann (1935) presented the following formula : -

$$ADP+CP \rightleftharpoons ATP+Creatine$$
$$AMP+CP \rightleftharpoons ADP+Creatine$$

Banga (1943) reported two kinds of enzyme catalysing these two reactions respectively and he purified the enzyme catalysing the first reaction. Recently, Narayanaswami (1952) studied the CPK system in brain and reported that the splitting of CP occurred rapidly in the presence of AMP. Namely, it occurs according to the reaction of Lohmann. When ADP was used in place of AMP the reaction was not so fast as with AMP and was still incomplete even at the end of 1 hour. From this result he concluded that the phosphate acceptor is AMP. On the other hand Ennor & Rosenberg (1953) reported that only ADP could function as an acceptor by using a purified enzyme of sheep muscle. In our experiment with the dialysed extract we recognized that not only AMP but also ADP could function as the phosphate-acceptor. But when the enzyme was purified according to the method of Lardy we found that the splitting of CP occurs only in the presence of ADP.

This result is in accordance with the view of Ennor & Rosenberg, so it may be decided that the phosphate-acceptor is ADP and the mechanism of the splitting of CP is expressed as following : -

$$ADP+CP \rightleftharpoons ATP+Creatine$$

It must be discussed, why AMP is able to take the place of ADP in the experiment of the dialysed extract. This means the existence of an enzyme such as Adenylate-kinase catalysing the following reaction : -

$$2ADP \rightleftharpoons ATP+AMP$$

Perhaps according to this reaction AMP will change into ADP and the activity of CPK will be maintained. But the investigation into this possibility will be a subject in future.

In regard to the reverse reaction, Narayanaswami reported that the concentration of the dialysed extract required ten times as much of that of the forward reaction and the speed of this reaction was slower, and in 40 min. it reached an equilibrium. Though we used the same concentration as in the forward reaction in our experiment, the reaction occured rapidly in the first 5 min. and thereafter the speed dilated and reached an equilibrium. Narayanaswami explained his result as an action of ATP-ase included in the extract. In order to inhibit the ATP-ase activity we added NaF in the reaction mixture but no effect was found. From our experiment it will be recognized that the speed of the reverse reaction is not so slow as Narayanaswami described. In regard to the pH optimum of the forward reaction, Lohmann described that the optima lay at pH 7.2-7.3, Narayanaswami pH 7.0, Lehmann pH 7.7, Ennor & Rosenberg pH 7.2. As to the reverse reaction Banga described that the optima lay at pH 9.05, Soreni & Degtyar pH 9.1, Lehmann pH 8.8, Ennor & Rosenberg more than pH 10.5. In our experiment in retina the pH optimum for the forward reaction lies at pH 6.8 and that for the reverse reaction at pH 9.0. From this fact, the activity of CPK is remarkably inclined to the forward reaction at the neighbourhood of the neutral pH. When ATP is decomposed the phosphate bond is immediatly provided from CP and it seems that CP is an amassed form of the high energy phosphate bond of ATP.

## Summary

1. In retina the activity of Phosphoamidase is almost negligible and the splitting of CP occurs owing to the CPK activity.

2. Using a purified enzyme we decided that only ADP could function as an acceptor of the phosphate bond of CP.

3. The optimal pH of the forward reaction lies at pH 6.8 and that of the reverse reaction lies at pH 9.0.

4. CPK is activated by $Mg^{++}$, slightly by $Ca^{++}$ but scarcely effected by $K^{+}$.

5. The activity of CPK is inhibited by the addition of $10^{-4}$ M PCMB and this inhibitory effect is completely released by GSH when it is added at the same final concentration as that of PCMB. Therefore we recognized that CPK belonged to a typical -SH enzyme.

6. Comparing the forward reaction with the reverse, this reaction inclined to the forward reaction at the neighbourhood of the neutral pH and consequently in retina as well as in muscle it is concluded that the phosphate bond of the decomposed ATP is provided from CP and the high energy phosphate bond of CP is consumed by the adenylic acid system.

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## THE INHIBITIVE ACTION OF GLUCURONIC ACID UPON THE DEVELOPMENT OF NAPHTHALENE CATARACT

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It has been known that glucuronic acid conjugates, as an important factor in detoxication, various toxic substances in vivo. Recently this drug has been widely used in the treatment of liver and skin diseases or fatigue. In this paper, it is reported that sodium glucuronate inhibits the development of a naphthalene cataract and the mechanism is discussed with respect to $\beta$-glucuronidase activity in the ocular tissues.

### Methods

Male albino rabbits weighing around 2 Kg. were used. A daily injection of 250 mg./gr. of naphthalene suspended in olive oil was implanted into the abdominal cavity. Some of the rabbits received the subcutaneous injection of 25 mg./kg. of sodium glucuronate* combined with naphthalene. The rabbits were divided into two groups. In the first group, naphalene alone, naphthalene with glucuronate or glucuronate alone was administered for 5 days in one group and in another group for 10 days. Twenty-four hrs. after the last injection, $\beta$-glucuronidase activity in various ocular tissues was determined by Talalay & Fishman's method and the development of the naphthalene cataract was observed by the usual clinical method. In the second group, naphthalene alone, naphthalene with glucuronate or glucuronate alone was administered and the enzyme activity was determined 24 hrs., 5,10 and 15 days after the last injection.

* The material was kindly presented by Chugai Pharm. Co. Ltd.

Enzyme activity was expressed in terms of phenolphthalein (in μg) liberated per hr. per gr. of the tissue from 0.01 Mol phenolphthalein glucuronide.

## Results

*The development of naphthalene cataract in the rabbits having been given naphthalene alone, or naphthalene and glucuronate for 5 and 10 days.* As seen in Table 1, glucuronate prevented the occurance and development of a naphthalene cataract.

Table 1. The Influence of Administered Glucuronic Acid upon the Development of Naphthalene Cataract in the Rabbits

| Drugs | Degree | Administration for 5 days | | Administration for 10 day | |
|---|---|---|---|---|---|
| | | Number | Total | Number | Total |
| Naphthalene | − | 3 | 3 | 4 | 4 |
| | + | 1 | | 3 | |
| | ++ | 0 | 3 | 1 | 6 |
| | +++ | 2 | | 2 | |
| Naphthalene and Glucuronic acid | − | 5 | 5 | 7 | 7 |
| | + | 1 | | 2 | |
| | ++ | 0 | 1 | 1 | 3 |
| | +++ | 0 | | 0 | |

*Variation of β-glucuronidase activity in the rabbit's eye of the first group.* Decreases in the enzyme activivity in the iris and ciliary body (Fig. 1) and choroid (Fig. 2) were caused by the administration of naphthalene alone for 5 days, the latter especially decreased to some 50% of the normal value and the activity of both tissues was restored to some extent

Fig. 1. Changes in the enzyme activity of the iris and ciliary body by repeated administration of drugs for 5 and 10 days.

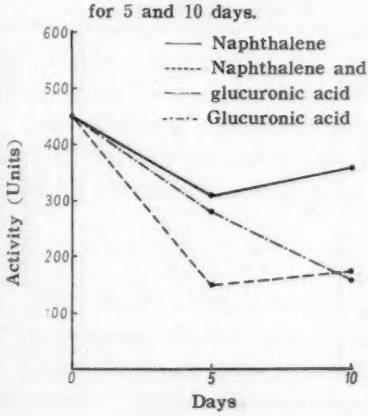
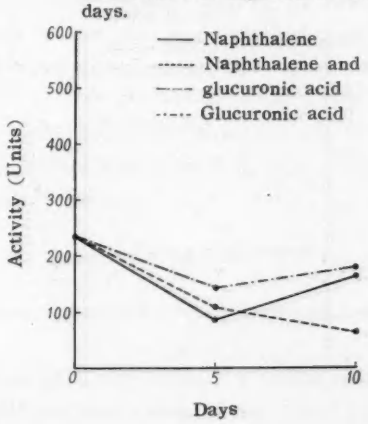



Fig. 2. Changes in the enzyme activity in the choroid by repeated administration of drugs for 5 and 10 days.



by the administration for 10 days. The enzyme activtiy in the retina (Fig. 3), lens (Fig. 4) and liver (Fig. 5) decreased in only a small degree, showing no remarkable changes. Combined administration of naphthalene and glucuronate for 10 days caused further lowering on all levels, this was brought on by the administration of naphthalene alone, especially regarding the enzyme activity in the ocular tissues. But this tendency is more remarkable in the iris and ciliary body than in the other tissues. The administration of glucuronate alone decreased the tissue $\beta$-glucuronidase activity to some extent, but the decrease was not beyond the decrease caused by the administration of naphthalene or naphthalene with glucuronate.

Fig. 3. Changes in the enzyme activity in the retina by repeated administration of drugs for 5 and 10 days.

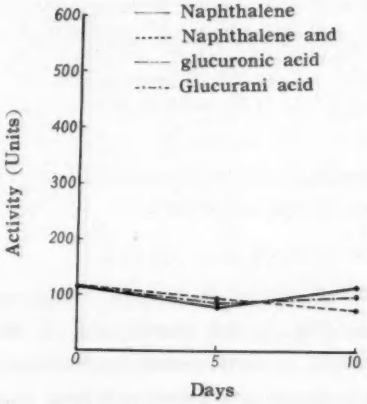



Fig. 4. Changes in the enzyme activity in the lens by repeated administration of drugs for 5 and 10 days.

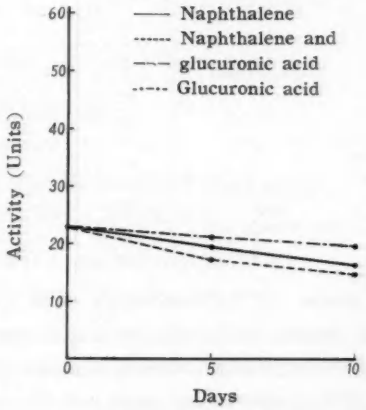



Fig. 5. Changes in the enzyme activity in the liver by repeated administration of drugs for 5 and 10 days.

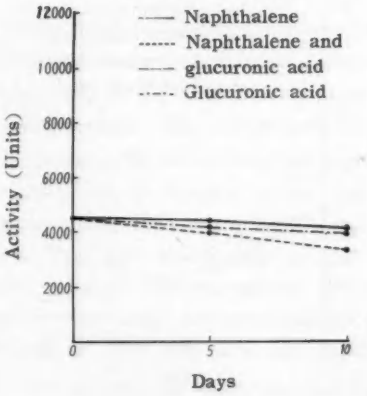



Fig. 6. Changes in the enzyme activity in the iris and ciliary body after repeated administration of drugs for 4 days

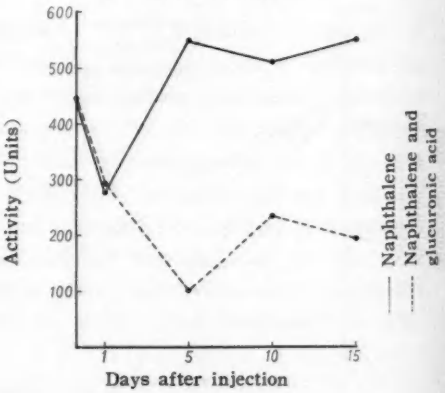


Fig. 7. Changes in the enzyme activity in the choroid after repeated administration of drugs for 4 days.

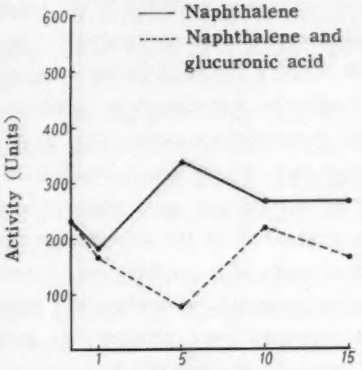
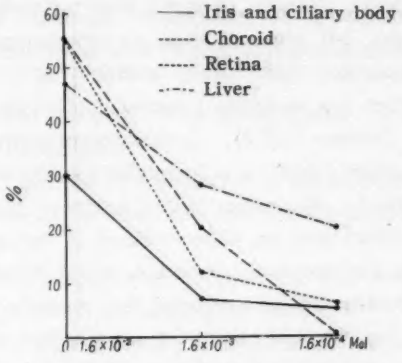



Fig. 8. The inhibition of glucuronidase activity in tissues by sodium glucuronate in vitro.




*Variation of β-glucuronidase activity in the rabbit's eye of the second group.* In the iris and ciliary body (Fig. 6), the enzyme activity decreased significantly after 24 hrs., and there followed a rise over the initial value after 5 days, the activity being still maintained after 15 days. The combined administration of naphthalene with glucuronate decreased the enzyme activity in the iris and ciliary body more in 5 days than in 24 hrs. and elevated the activity in 10 days, but the activity was far lower than the initial value. We were impressed with the fact that the variatien of the activity in the choroid (Fig. 7) was similar to that in the iris and ciliary body. In the retina, the enzyme activity rose in only a small degree following naphthalene administration, and decreased following naphthalene and glucuronate. In the serum, neither naphthalene alone nor the combined adiministration brought an appreciable change in the enzyme activity. It was found that in the liver β-glucuronidase activity an increase took place to some extent, 24 hrs. after the last injection, it returned to the normal value after 4 days, and again markedly increased after 15 days. The activity level brought by the combined administration of glucuronate was lower than that of naphthalene.

*The inhibition of β-glucuronidase activity in tissues by sodium glurcuonate in vitro* (Fig. 8). Out $1.6\times10^{-3}$ Mol glucuronic acid inhibited β-glucuronidase in the iris, ciliary body and retina, but affected the enzyme in the liver even more strongly.

## Discussion

It was found in this paper that the development of a naphthalene cataract was inhibited by the combined administration of glucuronate.

Corner & Young (1954) reported that naphthalene-dosed rabbits excreted following substances in their urine: 1-naphthol, 1-naphthylsulfuric acid, 2-naphthol, 1 : 2-dihy-

dronaphthalene-1 : 2 diol, 1 : 2-dihydro-2-hydroxy-1-naphthyl-glucosiduronic acid and 1-naphthylmercapturic acid. Ichihara (1953, 1954) extracted β-naphthoquinone, which was conjugated with mercapturic acid, in purified form from the urine of naphthalene-dosed rabbits. Namely, of naphthalene metabolites some substances were excreted in the isolated form, but most of them in the conjugate form with etheral sulfate, mercapturic acid or glucuronic acid. Many attempts have been made to identify the naphthalene metabolite which has an action inducing naphthalene cataract; 1-naphthol was studied by Igersheimer & Rubens (1910), 1,2-dihydroxynaphthalene, 1 and 2-naphthyl-glucosiduronic acid by Salffner (1904), and 2-naphthol by Kollinski (1889). All such efforts have resulted in a failure. Moriyama (1936) supposed that decreases in glutathione and cysteine in the cataract lens are closely related to the excretion of neutralsulfate in the naphthaleneurine; the increase in the excretion of this substance may be a cause of a naphthalene cataract. Recently, Ichihara reported that the occurrance of β-naphthoquinone in naphthalene rabbits is the essential cause of a naphthalene cataract, since cataracts were produced in guinea pigs of vitamin C defficiency by the injection of β-naphthoquinone extracted from the urine of naphthalene-dosed rabbits. However, the result obtained by Ichihara seems to show that some other substance than β-naphthoquinone should play a role in the production of a naphthalene cataract in the rabbits, even if it may be only as a supplemental factor.

It has been questioned whether supplied glucuronic acid enters into a glucuronide molecule. Packham & Butler (1954) and Douglas & King (1952) assured it by using $C^{14}$ labelled glucuronic acid or glucurone. From the above-mentioned results, it may be considered that an inhibitive effect of glucuronate on the development of a cataract is attributed to the role of glucuronate leading the metabolic pathways of naphthalene to produce the harmless glucuronides preventing the neutral sulfate conjugation or the production of substances causing naphthalene cataract.

It was also observed in this paper that the level of β-glucuronidase activity after naphthalene administration was markedly lowered by the combined supply of naphthalene and glucuronate, especially in the iris and ciliary body. Physiological role of β-glucuronidase has still remained unknown, but prevalent hypotheses were divided into following three groups.: 1. Increased β-glucuronidase activity is closely related to the cellular proliferation (Levvy). 2. Increased β-glucuronidase activity is not related to the cellular proliferation (Fishman, Mills). 3. On the contrary, this increase is related to the atrophy or damage of tissues (Pellegrino & Villani). Our knowledge about the physiological significance of the decrease in the enzyme activity is much more scanty than that of the increase. It may be, however, considered that the lowering action of glucuronate on β-glucuronidase in naphthalene rabbits is not only due to the inhibition seen in vitro, but also to some unknown action of glucuronate in vivo. Glucuronate lowering β-glucuronidase activity in the iris and ciliary body in vivo may prevent the isolation of aglucones, which are considered the toxic substances, from glucuronide. This prevention may produce a good result for the lens. It is supposed that from the two different causes mentioned above, the development

of a naphthalene cataract is inhibited by the administration of glucuronate.

## Summary

The inhibitive action of sodium glucuronate upon the development of naphthalene cataract was reported and the mechanism was discussed with respect to β-glucuronidase activity in the ocular tissue.

1. The iris and ciliary body and choroid showed the most remarkable change in β-glucuronidase activity after the administration of naphthalene.

2. The level of β-glucuronidase activity after naphthalene administration was markedly lowered by the combined supply of naphthalene and glucuronate, especially in the iris and ciliary body.

3. Injection of glucuronate alone inhibited the β-glucuronidase activity in the ocular tissues in vivo and it also inhibited the tissue β-glucuronidase activity in vitro.

From these results, the inhibitive action of glucuronate upon a naphthalene cataract was supported to be displayed in the following mechanism: glucuronate leads the metabolic pathways of naphthalene to glucuronide formation preventing its harmful metabolism and inhibits β-glucuronidase activity to isolate aglucones in the ocular tissues, especially in the iris and ciliary body.

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# THE EFFECT OF ATP ON THE CONE ACTION POTENTIAL

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## Introduction

It has been believed that the chemical change of the visual substances in the photo-receptors was accompained by electric potentials which transmitted the activities through the visual pathway. The energy of light can be exchanged for that of electric, by inducing the chemical change in the photo-receptors. The cone action potential which was found by Svaetichin in 1953[8], should be the potential generated by the photo-chemical changes as above. Accordingly, it may be supposed that the cone action potential is closely connected with such a chemical process as phosphorus metabolism, being responsible for the production of high energy. However, as far as it has been known, only fragmentary information is available on the effect of ATP upon the photo-receptor in the retina.

Therefore, the present experiments were undertaken in order to make clear the effect

of ATP on the cone action potential and to elucidate the relation between the cone function and phosphorus metabolism. In addition, this effect was compared with that of mono-iod-acetyl acid (MIAA) which had been known as an inhibitor of visual receptors.

## Material and Method

The eyes of carp (Cyprinus carpio linne, about 350 gr. in weight) were used as experimental materials. Each carp was sufficiently adapted to light at the beginning of all procedures. After the eye ball was excised out, it was cut through around the corneal limb, and the sclera and the pigment layer were removed carefully. The rods extending in the pigment layer following previous light adaptation were removed together with the pigments. But the cones contracting at the time of preparation remained intact and were exposed on the external limiting membrane. If the retina was placed with the receptor side upward in the container, the electrode tip could be directly applied to the exposed cone layer and inserted into the cell body without any difficulties. Two of these cone retina, which were obtained at the same time from the same carp, were placed individually in different containers filled with Ringer solution. These containers were kept in the dark in order to avoid the electric disturbances of the retina following the inadequate illumination and manipulation during procedures.

Then, the experiments were carried out in such a way that the cone action potentials were recorded from the two containers one after the other. The Ringer solution in one container was replaced with the 1/100 or 1/1000 Mol ATP Ringer solution, and the progressive changes of the cone action potentials in this solution were observed in contrast with those obtained from the other in Ringer solution.

An ultramicro capillary electrode filled with 3 Mol KCl, having a tip diameter of less than 0.5 micron and on electric resistance of about 20 MΩ, was employed as the recording electrode. A non-polarizable glass tube electrode containing 3 Mol KCl and filled with Ringer gelatin, was employed as the indifferent one. The former was inserted into a cone with the aim of a micromanipulator.

Light stimulation was supplied by a tungsten lamp with various filters. These filters were made by photographic films which were exposed to eight graded lights. The stimulus intensities obtained by using these filters were 10, 20, 50, 70, 95, 140, 200 and 300 lux, and the stimulus duration was about 0.7 sec.. After ascertaining that the cone potential was successfully led from a single cone inside, as mentioned in the previous paper[5], a series of responses to these eight graded lights was recorded in sequence every 1.5 sec..

The recording was made by a D.C. amplifier and a two-beam cathodray oscilloscope. The lower beam always displayed the phenomena, and the upper beam the time scale (1/5 sec.) and the signal of the stimulation.

## Results

The cone action potentials obtained in the present experiments showed several types such as the so-called usual type of potential consisting of simple hyperpolarization and

unusual types showing transiently overshots toward negative at on and toward positive at off, or showing only positive on-and off-response, etc.. Svaetichin has distinguished the cone action potentials into three types of B-Y and R-G cone, and L type cone in his recent paper on the spectral response curve of the cone potentials[10]. He proposed in this paper that the B-Y and R-G cones had to be the twin cones each of which consisted of blue and yellow cones, and of red and green cones, and that the L type cone had to be a single cone. From this point of view, it was suggested that the B-Y and R-G cones showed the unusual types of the potentials as above, and the L type cone the usual type of the simple hyperpolarization potential, when the retina was subjected to white light. The present experiments were carried out only on the usual type of potential which could be obtained more frequently than the unusual types of potentials. Changes in the amplitude, the rising and falling time and the on-latency of the potential, were measured.

*Effect of ATP.* Under the optimal condition in the Ringer container, the usual type of the cone potential ordinarily showed about 10 mV in amplitude, about 200 msec. in rising time and about 300 msec. in falling time, and about 17 msec. in latency, to the light intenaity of 10 lux. When the intensity reached nearly 150 lux to 200 lux, the minimum value, both of which remained approximately unchanged with varying intensities of more than 200 lux. But in the ATP-Ringer container, these values were gradually changed as one recording series was illustrated in Fig. 1.

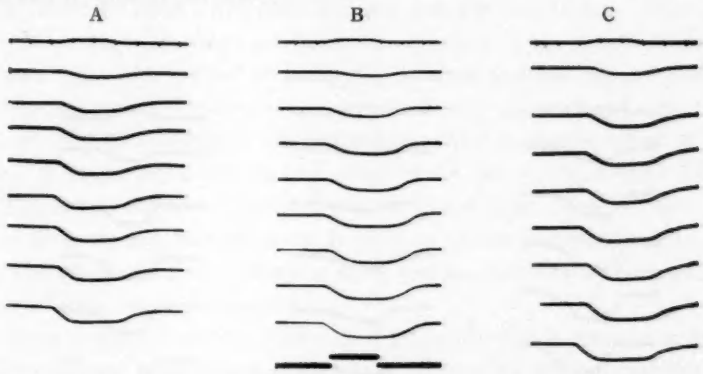


Fig. 1. A series of the cone potentials showing the effect of 1/100 Mol ATP solution; A obtained before, B and C after 5 and 20 minutes from administration, by using eight graded lights of 10 to 300 lux, as in the text, in sequence from up to down in each. Time, 1/5 sec.; Calibration, 10 mV.

The amplitude increased and reached the maximum value of 2 or 3 minutes after the administration of 1/100 Mol ATP solution. Then, the amplitude gradually decreased again approaching the standard value which was recorded at the same time in the other Ringer container, and after about 20 minutes both showed almost the same value. As the amplitude increased it was especially noted that amplitude further increased over the maximum value which was obtained in the standard Ringer container, and reached the maximum

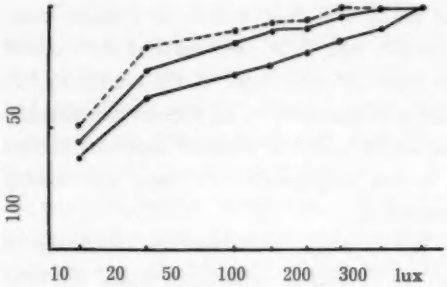


Fig. 2. The amplitude-intensity curves before (broken line), soon after (lower full line) and 20 minutes (upper full line) after administration of 1/100 Mol ATP solution.

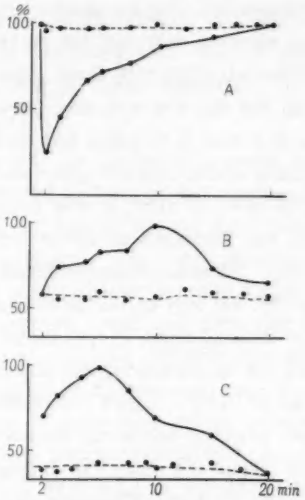


Fig. 3. Changes of the falling time (A). the rising time (B) and the on-latency (C) in minutes after administration of 1/100 Mol ATP solution (full line). Broken line showing the standard value.

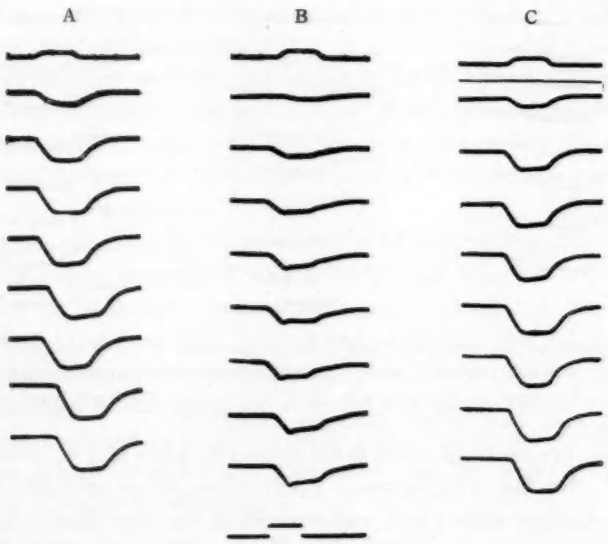


Fig. 4. A series of the cone potentials showing the effect of 1/10000 Mol MIAA solution, obtained before administration (A), 5 minutes after (B) and 20 minutes after (C) in the same way as in Fig. 1.

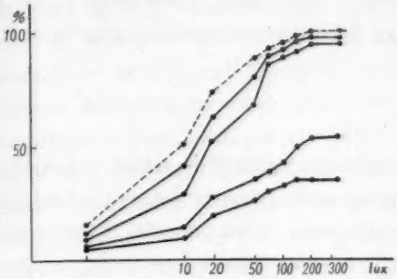


Fig. 5. The amplitude-intensity curves rbefore (broken line), 40 minutes after (upper full line), 20 minutes after (second), 10 minutes after (third) and soon after (lowest) administration of 1/10000 Mol MIAA solution.

value with the intensity of about 300 lux. Consequently, when the amplitudes were given in per cent each maximum value of amplitude measured in each individual recording series, the amplitude-intensity curves obtained after the adminwistration of ATP solution showed the downward deflection of more than those obtained from the standard retina, as illustrated in Fig. 2. Concerning this change, a little discussion will be made later.

Remarkable changes were observed on the rising and falling time and the on-latency. The relations between these changes and the minutes after ATP administration, obtained by the stimulation of a 10 lux light, were illustrated in Fig. 3,A,B and C.

The rising time and the on-latency began to increase immediatley after the administration of a 1/100 Mol ATP solution, and both showed the maximum values within 7 to 10 minutes. The maximum value of the prolonged rising time was about 340 msec. and that of the on-latency was about 30 msec., when the stimulus intensity was 10 lux. After reaching the maximum, these values gradually decreased and approached the standard values obtained in the Ringer container, within 20 minutes. Contrary to these changes, the falling time of the potential showed a paradoxical change as follows. It decreased abruptly soon after the 1/100 Mol ATP administration and reached the value of less than half of the standard one. Then, it gradually increased and returned to the standard value within about 20 minutes. The values illustrated in Fig. 3 were obtained from a case of light intensity of 10 lux. But these changes were observed in all cases of this experiment, being independent of varying intensities of light; almost the same results were also obtained when 1/1000 Mol ATP Ringer solution was administrated.

From these results, it may be clear that ATP inhibits the on-response of the cone action potential, but enlarges the brightness sensitivity of the potential and activates the off-response.

*Effect of MIAA.* The effect of MIAA upon the cone action potential was also observed in the same way as described above. The 1/1000 Mol and 1/10000 Mol Ringer solution, the acidity of which was previously canceled by adding a little amount of 1/1000 Mol NaOH solution, was used in this experiment. When the retina was soaked in 1/1000 Mol MIAA solution, the amplitude of the cone potential decreased markedly within 10 minutes; then, it increased gradually and about 20 minutes after being soaked, it almost recovered the standard value. A case of this experiment is illustrated in Fig. 4. The 1/1000 Mol MIAA solution produced the remarkable change of the irreversible reduction of the amplitude. After 20 minutes from its administration, it was frequently difficult to successfully record

the cone action potential. If the 1/10000 Mol solution was used, the change could be reversible as illustrated in Fig. 5. It is clear that MIAA suppressed the activity of the cone.

## Discussion

At present, some problems on the cone action potential remain to be solved. According to the general concept of neurophysiology, the hyperpolarization potential means the inhibitory action[2]. So, the first problem is whether or not the cone action potential is an exciting phenomena as expected in this paper. The second is whether or not it is really an intracellular potential. Tomita[12] proposed in his recent paper that this potential was not an intracellular cone potential, from the results obtained by measuring the depth of the intraretinal layer which showed the slow potential as mentioned above. However, it is considered that the receptor potential may not depend upon the concept of neurophysiology, because it is rather derived from the photo-chemical change.

Concerning the second problem, Svaetichin[9] has shown that the potential should be intracellular from the many facts which were confirmed again by the author[5]. Further, the author recently obtained the different results of measurement with Tomita's proposal[6][11]. From this point of view, the present experiments were carried out on the assumption that the hyperpolarization potential was exciting and intracellular cone potential, although these problems have not been solved[6,7].

It is well known that the excitement of photoreceptors is caused by the chemical changes of some photo-sensitive substances. These substances are considered to be rhodopsin, prophyropsin in the cone and iodopsin in the rod, which can produce the electrical phenomena by means of illumination even in vitro. Therefore, it can be supported that the cone action potential is also in association with more or less the photochemical changes of these substances. The usual type of cone potential showed hyperpolarzation during illumination and returned to the resting level following the cessation of light. This may suggest that the course of hyperpolarization coincides with the analytic phase of visual substances and the returning phase of the potential synthetic one. It is recognized by many research workers that the chemical substances which cause the rapid electric change in the retina are high energy compounds such as ATP, and that the energy originating by the analysis of ATP may be utilized for the synthesis of the photo-sensitive substances. An amount of phosphates analyzed during illumination has been found in the inner segment of the visual cell[1,4,8]. When the retina was soaked in the ATP solution, the falling time, which means the time course of the returning phase of the cone action potential, decreased, and the amplitude further increased following the increasing of light intensity than the maximum one, being observed in Ringer solution. These facts suggest the activating effect of ATP on the synthesis of the photo-chemical substances and the brightness discrimination of the visual receptors, giving support to the chemical observation concerning the role of ATP in the retina as described above. On the other hand, the on-latency and the rising time was

prolonged by ATP administration. This suggests the inhibitory effect of ATP on the on-response which is considered equivalent to the analytic phase of the photo-chemical substances. Mitarai and Yagasaki[5] found that the rising and falling time of the cone potential decreased by light adaptation and increased by dark adaptation. So far as the on-response is concerned, the inhibitory effect of ATP observed in this experiments, is similar to that of dark adaptation. But further work will be required to make clear these paradoxical changes of the cone potential produced by ATP administration. MIAA suppressed the amplitude and the course of on-and off- response. This inibitory effect is similar to the effect on the synthesis of visual purple which has been reported by Hwang[3] and others, and the contrary effect with of ATP.

## Summary

The effect of ATP on the cone action potential of Ciprinus carpio was observed under successive stimulation by a series of lights from 10 to 300 lux, and the effect of MIAA for the sake of contrast.

1. The falling time of the cone action potential decreased and the amplitude increased over the maximum value of the usual response after administration of ATP. On the contray, the potential was suppressed by MIAA. From these results it may be concluded that ATP activates the recovery mechanism of the cone function and increases its brightness discrimination.

2. The paradoxical changes were observed on the on-latency and the rising time. Both increased after administration of ATP. These facts were reserved to explain by further work in the future.

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# STUDIES ON THE GANGLION CELLS IN THE ROOTS OF THE 3RD, 4TH AND THE 6TH CRANIAL NERVES

## Report II

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The purpose of this communication is to study on the function of the ganglion cells which were descrided by the same author in his preceding report (Report 1). It was reported that there were some numbers of ganglion cells in the root of rabbit's ocular motor nerves, and the size of the majority of these cells varied from 25 to 32 micron in diameter. In all cases of the 3rd and the 6th nerve about 40 ganglion cells were counted, and judging from the arrangement of Nissl granules, the greater part of them resembled the sensory or autonomic cells. But the function of these cells could not be clarified completely in that report.

## Technique and Method

After the unilateral degeneration experiments, the rabbit's 3rd and 6th cranial nerves which contained these cells were studied histologically by the Nissl's staining method.

The operative procedures, used under ether anaesthesia, were cervical sympathectomy and the intracranial severance of the oculomotor nerve and the first division of the trigeminal nerve. The extirpation of the extrinsic eye muscles, the enucleation of the eye ball, and the ocular evisceration were carried out under the local anaesthesia in which the instillation of cocain solution was used. The oculomotor cutting experiment was carried out on 8 rabbits and the severance of the ophthalmic nerve was performed in 7 rabbits.

In these operations a midline incision was made at first, the skin reflected, the periosteum scraped back intact with a blunt instrument, the skull trephined on left side of the midline and some pieces of bone were removed from the cranial vault, being careful to separate the bone from the underlying dura mater. After the dura mater was reflected on both sides, some part of the frontal lobe were removed, and the brain was lifted on a smooth spatulate instrument until the root of the nerves oculomotorius or ophthalmicus became visible. The oculomotor nerve was cut with a iris hook and the ophthalmic nerve was contused with a diathermic needle completely.

Sympathectomy was performed in 7 rabbits. In this operation the skin of neck was reflected on left side of the midline and the superior cervical ganglion and the whole chain were removed.

In 6 rabbits the conjunctiva of the inferior part was cut, and the oculomotor nerve branches were severed from the inferior rectus or the inferior oblique muscles, and in a rabbit the abducens nerve branches supplying the external rectus were cut just before the insertion to this muscle.

The total extirpation of the inferior oblique and inferior rectus muscles was carried

out in 2 rabbits, and in other 2 rabbits the lateral rectus muscle was removed.

The ocular evisceration were carried out on 2 rabbits and in this operation the iris, ciliary body, cornea etc. were taken away from the eyeball, the entire ocular contents were emptied, thus and leaving only the sclera which was cleaned inside completely. The enucleation of the eyeball were tried in 2 rabbits moreover. Most of these animals were kept alive for 7, 10 and 15 days, but when the oculomotor or ophthalmic nerve were cut, these rabbits were kept alive for 2, 3, 5, 7, 8, 10, 11, 15 and 20 days. In the sympathectomy the lapse allowed for degeneration was 10 to 34 days.

The rabbits were killed with 70 per cent alcohol solution which were injected into the heart. As quickly as possible the cranial vault was taken away from the head and the roots of the 3rd, and the 6th cranial nerves were removed. These nerves were fixed for about 3 weeks in a solution of 96 per cent alcohol, embedded in celloidin, cut in serial section, and stained with thionin to examine the chromatolysis in the ganglion cells in question.

## Observations

After the unilateral degeneration experiments, the ganglion cells which were contained in the 3rd and the 6th cranial nerve roots were studied histologically.

The findings are as follows:

1) By the intracranial severance of the oculomotor or of the ophthalmic nerve, these ganglion cells did not show any sign of degeneration.

2) When the nerve branches supplying the extra-ocular muscles were cut just before their insertion to the muscles, chromatolysis was not observed in these cells.

3) When the superior cervical ganglion and its chains were removed, these cells were reserved intact.

4) When the ocular evisceration were carried out, these cells exhibited no departure from the normal appearance, but by the enucleation of the eyeball, the chromatolysis was encounted in these cells.

5) When the inferior oblique as well as the inferior rectus muscle or the lateral rectus muscle was removed, the degenerating reaction was found in these cells.

From the foregoing description, it is evident that the ganglion cells located in the roots of the rabbit's oculomotor nerve are unipolar cells and probably concerned with the innervation of the extrinsic eye muscles.

## Discussion

The results of the severance of the oculomotor or the ophthalmic nerves and sympathectomy suggest that these ganglion cells do not belong to the afferent neurons, because it is not expected that there is other pathway of these neurons. And the efferent neurons of these cells are not contained in the cranial nerve branches supplying the extra-ocular muscles, because these cells were reserved intact after these nerve branches were cut

before they enter the muscles.

It could be explained that these cells innervate the extrinsic eye muscles themselves other from the tissue of the eyeball, by means of the datas of the ocular evisceration and the total extirpation of some of the extra-ocular muscles. The finding of the ennucleation of the eyeball is understood that the neuron of these cells were injured by this operation. Before the function of these cells were discussed, the data which were described by the same author in his report on the nerve endings in the extrinsic eye muscles of rabbit must be refered.

The conclusion of that report are as follows:

1) In the extrinsic eye muscles of the rabbit, there are 6 types of the nerve endings.

2) In these endings, the nerve endings originating from thick medullated nerve fibers and from thin medullated nerve fibers are contained, and the former apparently represent the motor end plates of Kühne and the latter correspond with the atypical motor ending of Retgius and seems to be sensory nerve ending.

3) When the oculomotor nerve trunk was cut at the exit from the brain, both these nerve endings and their supplying nerve fibers degenerated completely, but by the intracranial severance of first division of trigeminal nerve just distal to Gasserian ganglion, all nerve endings and fibers exhibited no departure from the normal appearance.

4) In case when the superior and medial cervical ganglion and its chains were removed, the orbital muscles and the palpebral smooth muscles (Müller's muscle) were denervated completely.

These findings may indicate that the nerve fibers which innervate the extraocular muscles originate from both the ocular motor nerve and the cervical sympathetic, and the nerve fibers of the motor and of the sensory were incorporated in the nerve trunk of the ocular motor nerve. This conclusion does not contradict to the datas of the present work; and then these ganglion cells are not concerned in the motorial and sensorial innervation of the extrinsic eye muscles.

On the other hand these datas gave evidence that these cells do not innervate the tissues of the eyeball or the orbital muscles and the palpebral smooth muscles.

In the orbit there are the lacrimal gland, the Harder's gland and the orbital fat with the exception of extrinsic eye muscles, eyeball, and the orbital and palpebral smooth muscles. It is certain that these cells are concerned with the innervation of the extrinsic eye muscles, because of the present position of these cells and the results of the total extirpation of the extra-ocular muscles.

Sunaga ('27) and Kojima ('28) investigated the trophic innervation of the eye muscles and reported that the extrinsic eye muscles exhibited atrophic appearance by extirpation of the ciliary ganglion. Kojima noted that the sympathic nerve also was concerned with the trophic innervation of the eye muscles.

Therefore it may be concluded that these cells are the autonomic nature like those of the ciliary ganglion and probably concerned with the trophic innervation of the extrinsic eye

muscles.

In the rabbit it is almost impossible that these cells are removed from the ocular motor nerve trunk without the compulsion of the other nerve fiber, because of the position on which these cells are distributed. According to this fact, the extirpation of these ganglion cells could not be carried out in the rabbit.

## Summary

Unilateral degeneration experiments were carried out on 37 rabbits and with the lapse of from 2 to 21 days those animals were killed, and the 3rd and the 6th cranial nerves were stained with thionin to examine the chromatolysis in the ganglion cells located in these nerve roots.

By the intracranial severance of the oculomotor or of the ophthalmic nerve and the sympathectomy, these ganglion cells did not show any sign of degeneration. When the nerve branches supplying the extra-ocular muscles were cut, just before their insertion to the muscles, and the ocular evisceration were carried out. these cells were reserved intact.

When the inferior oblique as well as the inferior rectus muscle or the lateral rectus muscle was removed, typical chromatolysis was encountered in these cells.

By the enucleation of the eyeball chromatolysis was observed in some of these cells, because the neurons of these cells were injured by this operation.

It may be concluded from the present and previous reports that these cells are probably concerned with trophic innervation of the extrinsic eye muscles.

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Fig. 1. A ganglion cell in the root of the 3rd cranial nerve on the left (n. oculomotor sectioned). Taken from the left 3rd cranial nerve, 11 days after cutting the oculomotor nerve and this cell is reserved intact (Nissl's staining). (The same appearance was found also after cutting the ophthalmic nerve and sympathectomy.)

Fig. 2. A ganglion cell in the root of the 3rd nerve on the right (unoperated) side. Taken from the same rabbit in figure 1.

Fig. 3. A ganglion cell in the root of the 6th cranial nerve on the left (ocular eviscerated). Left ocular evisceration, 15 days. This cell show the appearance of the typical chroma tolysis and the displacement of the nucleus.

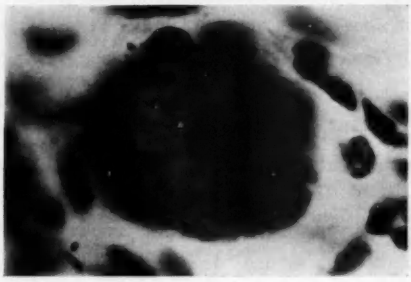


Fig. 4. A ganglion cell in the 6th nerve root of the right (unoperated) side. Taken from the same animal as in figure 3.

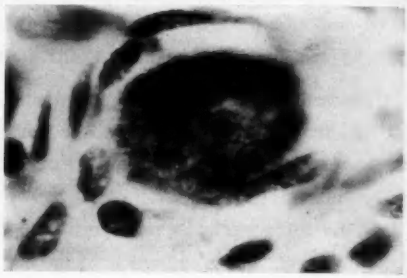


Fig. 5. A part of the left ciliary ga nglion. Left enucleation of the eyeball, 15 days.

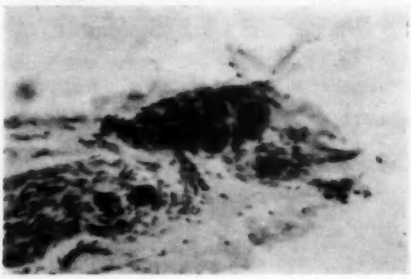


Fig. 6. The magnified appearance of these cells in figure 5. The nucleus of all cells displace and right ganglion cell is swelling.

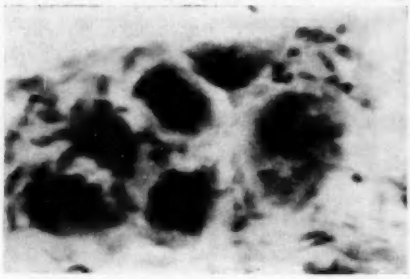


Fig. 7. A ganglion cell in the root of the 6th cranial nerve on the left. Left lateral recus muscle extirpation, 15 days. Its nucleus is pushed aside and its granules are degenerated.

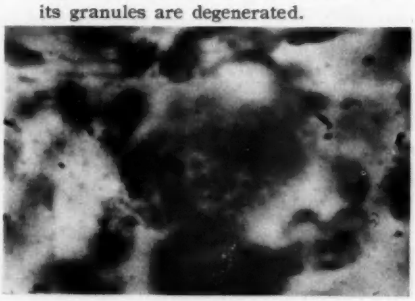
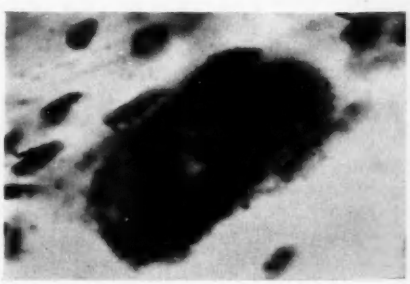


Fig. 8. A ganglion cell in the root of the right 6th nerve of the same animal as in figure 7.



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# OBSERVATION OF VISUAL CELLS BY MEANS OF PHASE CONTRAST MICROSCOPY

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Fresh visual cell of the retina were studied from the end of the last century until the beginning of this century; early experinents with frogs, in particular, were fully described by Gaup (1904). During the last five decades the limited number of reports was mainly concerned with fixed materials; these were reported by Majima (1922), Ohashi (1926), Matsuura (1933), Schmidt (1934), Uyama (1951) and others. Recently, the fine structure of visual cells was revealed by Sjostrand (1953), this progress can be attributed to the advance of electron microscopy.

This study is concerned with the structure and morphological changes of live frog's visual cells by means of phase microscopy.

## Methods

Adult bull frogs were used in the experiments. Before test took place, the animals were kept in the dark for 12 to 24 hours, after which they were decapitated. Immediately after the eye was removed, the retina was excoriated from the pigment layer and was dissected as thin as possible with two ophthalmological knives. This section was enclosed by physiological saline or the vitreous body of each animal. Then these specimens were examined by Leitz's phasem icroscope which was equipped with a green filter. Köhler's light, manufactured by Olympus OpticalCo, was applied. A Zeiss-type water tankfilter was used as a protection against the light bulb's heat rays. Microphotographs were taken by Photorest (manufactured by Chiyoda's Optical Work) which is an attachment for the 35 mm Canon camera.

In order to compare the fresh materials with the fixed ones, the new specimens were prepared by Green's method (1950) with modifications by Mizuhira's (1952).

## Results

Each layer of the retina was easily distingushed, because it was cut in relatively thin sections. Abundant rods and a few cones, being isolated from the specimen at the inner or outer segment, floated all around the section. The fine structure of both visual cells was fully observed.

*Rod* : *Outer segment*. When Heine's condenser of Leitz's phase microscope was adjusted to the dark field, an outer segment of rods, coated with a homogenous membrane of "neurokeratin", and showing a symmetrical cylinder in which its distal end represented a dome (Fig. 1) could be seen. Immediately after enclosing, some stripes which run parallel to the longitudinal axis of a rod, were observed on the surface of the outer segment (Fig. 2). These are thought to be shallow ditches. After that, the outer segments began to extend; at the same time, the longitudinal stripes gradually disappeared, and other dense transverse stripes appeared (Fig. 3). As time went by, these transvese stripes became clearer and clearer, till at last they presented a spiral cord just like a nicrome wire (Fig, 4).

The most characteristic change of rods was the "curling phenomenon" near the distal part of the outer segment; viz., the rods began to curl immediately after being mounted, and about 30 minutes later, they took the shape of brackens (Fig. 5). Especially as soon as the rods were enclosed in the hypotonic solution, such as 0.32% NaCl sol., this phenomenon was obviously observed.

*Rod : Inner segment*. The inner segment was distinctly divided from the outer segment by a membrane which strongly reflects the light in fresh sections (Figs. 6 and 7).

From the morphological point of view, two types of inner segments were observed in frogs; the one was the cylinder type, the other the cone type. An inner segment of the cone type looked like a Japanese writing brush, and showed homogenous structure. Namely, this segment had no ellipsoid containing any granules or fibers (Fig. 6), while, that of the cylinder type had a half spherical ellipsoid in which abundant granules or Golgi-like nets of fibers were observed (Fig. 7), and at first showed a cylinder, however easily swelled up to a globe.

*Cone*. The outer segment of a cone was smaller than that of a rod, and was observed as a fragilable process. A thick and loose spiral was also found within the segment. The inner segment showed as oval form and contained a fat globe which strongly reflects the light. Other granules could not be found anywhere (Fig. 8).

The present observation could not reveal so-called outer or inner filament which was observed by Kolmer (1904) in both rods and cones.

## Discussion

Concerning the longitudinal stripes on the surface of the outer segment in the frog, Schultze (c.f. Gaup) observed them by means of an optical microscope in fresh materials, and considered that they formed fine ditches on the outer segment. Hensen (c.f. Gaup) recognized the fibers between these ditches and said there were 24 fibers per rod. Merkel

(c.f. Gaup) supposed that these ditches were caused by the mechanical pressure due to the pigment migration; therefore, projection of the pigment epithel descended along these ditches. Uyama (1951) also observed longitudinal or transverse fibers in both rods and cones, but he considered that these fibers must be artificial products owing to procedures of fixation. The present observation revealed that some longitudinal stripes were observed on the surface of the outer segment only when the specimens were fresh. These are considered shallow ditches.

There are some questions as to whether the outer segment of rods shows a spiral structure or a pile of discs. Krause (c.f. Gaup) observed a spiral fiber within the outer segment by the treatment of 1% Lysol solution; Hesse (c.f. Gaup) also found the same phenomenon in the frog. Majima (1922) assured the existence of a spiral cord within the outer segment on smear preparates which were treated with the dilute chromic acid solution for a few minutes. Sjostrand (1948, 1949 and 1953) has reported on the structure of the outer segment of the guinea pig or perch by means of electron microscopy. He stated that the outer segment was composed of a pile of double membrane discs, and the oriented lipid molecules were localized to the compartment between the membranes of the discs. Our continuous observation by means of phase microscopy revealed that the transverse stripes appeared after the longitudinal stripes went out of sight; at first these stripes looked like a pile of many discs, but, with the extension of the outer segment, they gradually changed into a spiral cord which looked just like a piece of nicrome wire.

The observation of the "curling phenomenon" of the distal end of the outer segment could not be found in any descriptions so far as we know. Uyama (1951) has supposed that the permiability of the distal end is higher than that of the other part. He based his theory on his observation of some rods which showed various clavate forms in the outer segment on the fixed preparates. However, we were unable to observe such figures in fresh materials. We have surmised that the distal part of the outer segment will probably be differnt from the other part in the nature, and the "curling phenomenon" will be correlated with the spiral structure within the outer segment.

Two kinds of rods in the frog were described by Gaup; the one is the red rod, being 80$\mu$ in length and 6$\mu$ in breadth, and the other is called the green rod which was found by Schwalbe (c.f. Gaup), being 34.5$\mu$ in length and 6.125$\mu$ in breadth, having a thread-like inner segment in which the distal part expands like a cone, and attached to the outer segment. The present observation also confirmed these two kinds of rod; the red rod is thought to be the cylinder type, having a distinct ellipsoid which contains abundant granules or the nets of fibers, and the green rod corresponds to the cone type in which no structure can be found within the inner segment.

Between both segments, there is a partition plate which is called the "Kittsubstanz" by Schultze (c.f. Gaup) or the "Zwischenscheibe" by Greef (c.f. Gaup), and both segments are easily separated from each other at this place. This plate was observed as the substance which reflects the light.

Regarding the structure of the cone, Sjostrand observed the outer segments of perch cones, and he stated that the outer segment was composed of a pile of single membrane discs in which these edges were slightly thickened. In our observation of the frog, the spiral structure was revealed within the outer segment.

## Summary

This observation is concerned with the structure and the morphological changes of visual cells of the bull frog on fresh condition by means of phase microscopy.

1. Immediately after mounted in the physiological saline or the vitreous body, some longitudinal stripes were observed on the surface of the outer segment of rods. These stripes seem to be shallow ditches.

2. In the course of time, the longitudinal stripes became indistinct, as the outer segment extended, and other dense transverse stripes appeared. These gradually became clearer and at last presented a spiral cord just like a piece of nicrome wire.

3. The most characteristic change was the "curling phenomenon" near the distal part of the outer segment. Especially, as soon as they were enclosed in the hypotonic solution (i.e. 0.32% NaCl sol.), the rods took the shape of brackens.

4. Two kinds of inner segment of the rod were observed in the frog; the one showed a cylinder type and had an ellipsoid which contains abundant granules or nets of fibers, although this inner segment easily swelled up to a globe. The other presented a cone form which lacks an ellipsoid; therefore, no structure could be found within the inner segment.

5. The structures of both outer and inner segment of the cone were observed distinctly as well as those of the rod: the cone-shaped outer segment had also a spiral cord which is thicker than that of the rod, and the oval-shaped inner segment contained a fat globe which reflects the light.

(Published in Acta Ophthalm. Jap. 61: 918, 1957 in Japanese)

Fig. 1. The fresh section of the frog retina under the dark field. The outer segment of the rod is coated with a membrane of the "Neurokeratin" and the distal end shows a dome. 10×5

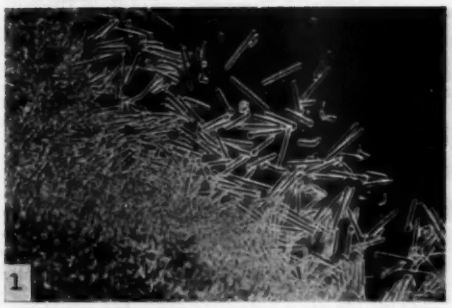

Fig. 2. The longitudinal stripes on the surface of the outer segment of the rod immediately after being enclosed. 40×10

Fig. 3. The transverse stripes which appear after the longitudinal stripes have gone out of sight. 40×10

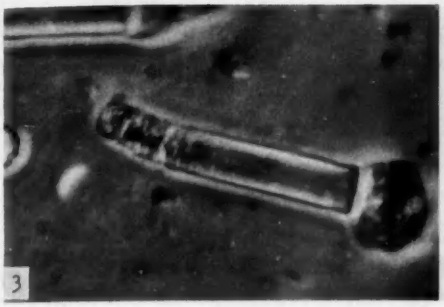

Fig. 4. The spiral structure within the outer segment of the rod. 40×10

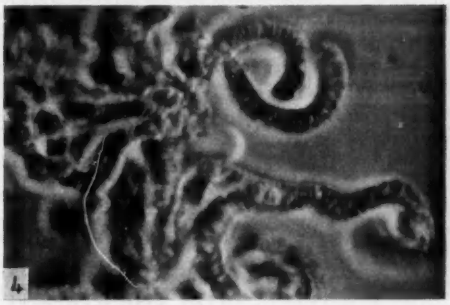

Fig. 5. The "curling phenomenon" near the distal part of the outer segment of the rod, being enclosed in the hypotonic solution. 20×5

Fig. 6. The inner segment of the cone type. 40×10

Fig. 7. The inner segment of the cylinder type, which has an ellipsoid having some granules or the nets of fibers. 40×5

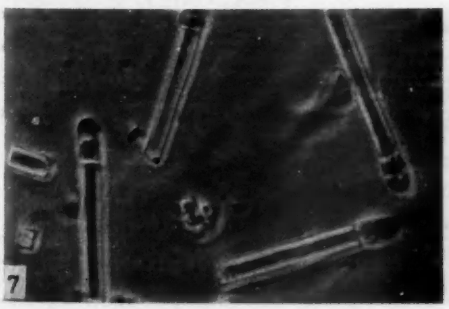

Fig. 8. The cone having a cone-shaped outer segment and an oval inner segment which contains a fat globe. 40×10

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## PHASE-CONTRAST MICROSCOPY OF THE VITREOUS REPORT III THE EFFECT OF HYALURONIDASE ON THE STRUCTURES OF THE VITREOUS

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In 1952 Bembridge, Crawford and Pirie attempted to study under the phase-contrast microscope and found coarse branching fibers in the base, fine parallel fibers in the center and hyaline membrane on the surface of the vitreous body.

The author studied the fibrous structures of vitreous in oxen, dogs and rabbits under the phase-contrast microscope and found similar fibrous structures to those observed by Bembridge. The effect of hyaluronidase on fibrous structures of the vitreous was studied further under the phase-contrast microscope in the following experiment.

### Methods

First the effect of hyaluronidase on the fibrous structures of the vitreous was examined in vivo ; then the digestibility of hyaluronidase on those was examined in vitro.

The hyaluronidase preparation used in this experiment is "Harodase" (Takeda) containing 5000V.R.U./ampule.

A salt solution of hyaluronidase usually was used. Dogs of either sex between 2 and 4 months in age were used. After the hyaluronidase solution containing 5~100 V.R.U. was injected into the vitreous and a solution containing 250-5000V.R.U. was injected subconjunctivally, the eyes were removed at regular intervals after the injection and the pathological changes of the fibrous structures of the vitreous were immediately studies under a phase-contrast microscope. A hyaluronidase solution containing 5-100V.R.U./cc. was used in the experiment of digestibility in vitro. Phase-contrast microscopy was done in positive phase in this experiment.

### Results

*Effect of Intravitrear Injection of Hyaluronidase.* A hyaluronidase solution of 0.1-0.2 cc. of various concentration were injected into the vitreous, while the eyes were removed at regular intervals after the injection and the fresh vitreous was examined under a phase-

contrast microscope.

1) Injection of Hyaluronidase of 50-1000V.R.U.

An injection of 50-100V.R.U. caused an intensive inflammatory reaction in the eyes in 24-72 hours after the injection: hemorrhage and fibrous exudate appeared in the anterior chamber and in the vitreous body, while the pupil was occluded with exudate. Two to three weeeks after the injection the exudative inflammation began to decrease. The liquefaction of the vitreous was intensive after 2-3 weeks.

Findings under the phase-contrast microscope: The fibrous structure of the vitreous could not be seen because of the appearance of many inflammatory cells (red blood cells, round cells, ciliary cells and ciliary pigment granules) 48 hours after the injection; but after 72 hours those cells began to disappear and the changes of the fibrous structure of the vitreous could be seen gradually. A partial degenerative swelling and a fine network building were observed in the base of the vitreous. Many pigment granules of the ciliary body were seen in those networks. The fibers ran irregularly. The aspects of the fibrous membrane of the surface of the vitreous were not clearly seen and showed a partial tear.

After 2-4 weeks still some inflammatory cells remained. The density of the fibrous bundles became coarse and a fine network building with many pigment granules was seen in the base of the vitreous. The tear and the fine network were observed markedly in the membranous structure of the vitreous surface.

2) Injection of Hyaluronidase of 30-40V.R.U.

The inflammation after the injection was not so severe. Twenty four to forty-eight hours after the injection a hemorrhage and fibrous exudate appeared usually in the anterior chamber, and then the inflammation disappeared gradually 1-3 weeks after the injection. Intensive liquefaction of the vitreous was observed after 3 weeks.

Findings under the phase-contrast microscope: After 48 hours the fibrous structure was able to be observed in both regions of the vitreous. The aspects of the fibrous structure were not still clear in detail. Numberless inflammatory cells and red blood cells were seen in those fibrous structures. Ninety-six to one hundred and twenty hours after the injection, a partial degenerative swelling of the fibrous was able to be observed in both regions of the vitreous.

Three to four weeks after the injection, the fibrous became irregular and degeneration of the fibrous became more intensive in the base. The membranous structures became more indistinct and fine networks were seen in the surface of the vitreous.

3) Injection of Hyaluronidase of 10-30V.R.U.

A slight reactive inflammation occurring 24-48 hours after the injection, disappeared 96 hours-1 week after the injection.

Findings under phase-contrast microscope: 24-48 hours after the injection the fibrous structure of the base became considerably more clear and the fiber ran quite normal as usual.

The reaction of the membranous structure of the surface was not clearly visible; many fine network and little inflammatory cells were seen.

After 3 weeks part of the fibers of the base showed degenerative swelling, while most of them were normal. In the membranous structure of the surface many fine networks were observed.

4) Injection of Hyaluronidase of 5 V.R.U.

A reactive inflammation was hardly seen in the eyes, and sometimes a slight inflammation of the cornea appeared transitorily 24-48 hours after the injection. Neither exudate nor hemorrhage was seen in the anterior chamber or the vitreous. Liquefaction of the vitreous was observed rarely after 2-3 weeks.

Findings under the phase-contrast microscope: The fibrous structure of the base showed no pathological change for 2.5 months. In the membranous structure of the surface a slight degenerative swelling and a slight fine network building began to appear 96 hours-1 week after the injection. Those changes became slighter after 2.5 months.

5) Control Examination.

After a 0.1-0.2 cc. saline solution was injected into the vitreous, the eyes were examined in the same way. No pathological change was observed microscopically. Sometimes a temporary rise of tension caused a corneal edema and mydriasis, which disappeared within 24 hours.

*Effect of Subconjunctival Injection of Hyaluronidase.* A 0.5-1.0 cc. solution of hyaluronidase was injected subconjunctivally and the eyes were removed after 24-48 hours. The preparations from a fresh vitreous were examined under the phase contrast microscope.

The subconjunctival injection of a hyaluronidase solution caused a slight chemosis for 24-48 hours and only the injection of 5,000 V.R.U. transitorily caused a slight iritis and a partial synechisis of the iris.

Findings under the phase-contrast microscope: After injecting 250-2,000 V.R.U. no pathological change was found in the fibrous structure of the vitreous; by injecting 5,000 V.R.U. a fine network building appeared slightly in the membranous structure of the surface.

*Effects of Hyaluronidase in Vitro.* A piece taken from a fresh vitreous body was immersed in a salt solution of hyaluronidase and the mixture was incubated at 37°C., for 72 hours. The preparations from a piece incubated were examined under the phase-contrast microscope.

In the solution containing 500V.R.U./cc. the form of the vitreous vanished microscopically and only a part of the degenerative fibrous structures in the base was observed with difficulty under microscope.

In the solution containing 250V.R.U./cc., a trace of the degenerative structure was observed microscopically, but its origin could not be decided upon because the degeneration was so marked. The other changes were the same as the former experiment.

In the solution containing 100V.R.U./cc., the vitreous became smaller and its viscosity

decreased. A few degenerative fibers remained in the base, while the membranous structure disappeared entirely on the surface of the vitreous.

In the solution containing 50V.R.U./cc., the form of the vitreous was kept well, while its viscosity decreased slightly. The fibers of the base could be seen considerably, but the degenerative changes were intensive. The membranous structure was not distinct and a few irregular fiber were observed in then.

In the solution containing 5-20V.R.U., the form of the vitreous did not change and its viscosity was normal. The fibers of the base were kept normal, but the membranous structure of the surface was not distinct and showed a partial fine networkbuilding. In the controlled experiment most fibers of both regions of the vitreous appearked normal but partially degenerative.

## Discussion

L. von Sallmann (1944) pointed to the fact that the diffusion of penicillin in the vitreous was slow. Pirie, Schmidt and Water (1948) suggested that fibrillen protein networks were immersed in hyaluronic acid of the vitreous and that those compositions presented the diffusion.

The effect of hyaluronidase on the vitreous was studied by means of examining whether hyaluronidase injections facilitated the diffusion in the vitreous or not. L. von Sallmann (1948) said that a hyaluronidase injection accelerated the diffusion of red blood cells in the vitreous of rabbits. He (1949) described that the mixtures of hemoglobin or dye with hyaluronidase did not diffuse rapidly in the vitreous but the diffusion was increased by temperature increases. He (1950) also recognized that high concentration of hyaluronidase facilitated the disappearance of red blood cells in oxen vitreous because the phagocytosis was increased by causing inflammation in the vitreous He also suggested that a rabbit's vitreous contained less hyaluronic acid than an ox's vitreous, therefore the diffusion in a rabbit's vitreous was more rapid than in an ox's vitreous.

Pirie (1949) also said that the filtrate of vitreous was affected immediately by hyaluronidase but the vitreous remaining intact was affected only gradually.

The inflammation caused by an injection of hyaluronidase into the vitreous was observed by many students.

The author studied the effects of hyaluronidase on the fibrous structures of the vitreous under a phase-contrast microscope in those experiments.

The injection of a high concentration of hyaluronidase (30-100 V.R.U.) into the vitreous caused a strong inflammation in the eyes as many men pointed out.

The fibrous structures of the vitreous could not be observed in a short time after the injection because those were hid by exudate. In 2-4 weeks after the injection, the author found a partial degenerative swelling of fibers in the base, a tear building; a fine network building was obsered in the membranous structure on the surface. It seemed that not only the act of hyaluronidase, but also the inflammation caused by the polypeptid of an

enzyme resulted in these pathological changes. But in the cases of the injection of low concentration of hyaluronidase (5-20 V.R.U.), only a slight reactive inflammation was caused transitorily and the membranous structure of the surface was not seen clearly, where the fine network building was seen. Both those results in these experiments show clearly that the inflammation did not cause the pathological changes in the vitreous structures, but the hyaluronidase injection itself. Therefore the author thinks that the membranous structure of the surface was made of fine fibrillen networks which were full of hyaluronic acid, and that a hyaluronidase injection of decomposed hyaluronic acid and fine fibrillen networks remained here.

J.T.H. Planten and R. Hoppenbrowers (1954) observed the turbidity in the anterior chamber and in the vitreous after the intraocular hyaluronidase administration in rabbits. They said that those changes were not due to an inflammation but due to results of an enzyme.

L. von Sallmann and Price stated that hyaluronidase injections had no effect on the intact vitreous but on only the filtrate of the vitreous. In their experiments the removed eyes were used as material; the diffusion into the vitreous was oberved microscopically. But in these experiments, the author used the vital eyes and observed the changes of the surface structure by phase-contrast microscopy with in 24-48 hours after the injection of a low concentration of hyaluronidase. The results obtained in these experiments showed that hyaluronidase injections diffuse rapidly in the vitreous. On the other hand, the fibrous structure of the base had the degenerative swelling with strong inflammation after the injection of a high concentration of hyaluronidase, but they showed no change after the injection of a low concentration of hyaluronidase. The results of this experiment show that a hyaluronidase injection brought little change in the base.

According to Meyer, hyaluronic acid is produced in the ciliary body and flows into the anterior chamber. Thus the concentration of hyaluronic acid seems to be higher in a ciliary region of the vitreous. The author supposed that hyaluronidase injections acted upon hyaluronic acid in the base and were discharged quietly from many blood vessels of the ciliary body, and therefore that hyaluronidase had little effect on the fibrous structure in the base.

Next, the subconjunctival hyaluronidase injection resulted in weaker changes. Only in the high concentration hyaluronidase injection (2,00-5,000V.R.U.) the author observed a little fine network building on the surface of the vitreous. The spread-like factor of hyaluronidase diffuses rapidly in subconjunctival tissue and a part of hyaluronidase invades the vitreous, so that it effects only slightly the fibrous structures of the vitreous.

Finally the author observed the liquefactive effect of hyaluronidase in the vitro. In the cases of high concentration, the membranous structures of the surface disappeared entirely and the fibrous structure of the base showed a considerable degeneration.

On the other hand, low concentration of hyaluronidase brought no strong changes in the base or a fine network building in the surface structures. These results showed that

Fig. 1. One week after injection of 50 V.R.U. Tear building in the membranous structure of the surface (×400).

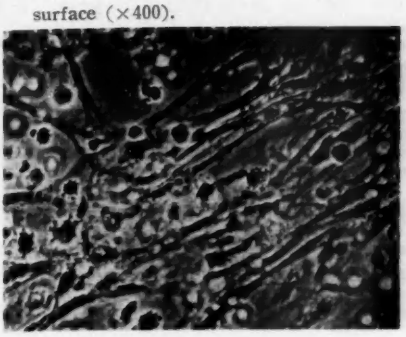


Fig. 2. 4 weeks after injection of 50 V.R.U.. The fibers of the base run normal, and a fine network building are seen partially (×400).

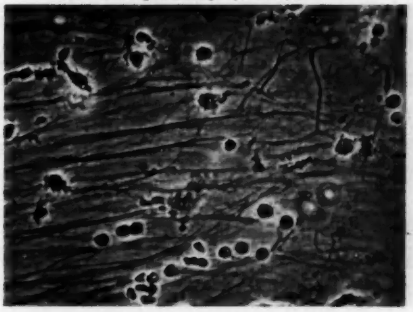


Fig. 3. 4 weeks after injection of 20 V.R.U.. Membranous structure of the surface disappears and fine network buildings are distinct (×400).

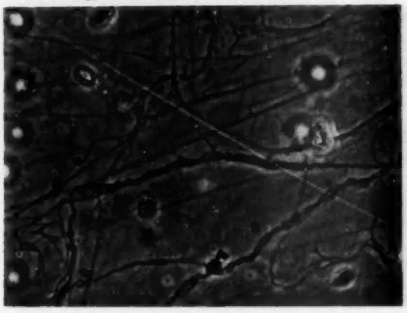


Fig. 4. 4 weeks after injection of 5 V.R.U.. The membranous structure of the surface is irregular and a partial fine network building is seen (×400).

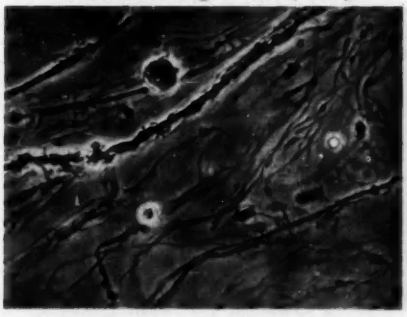
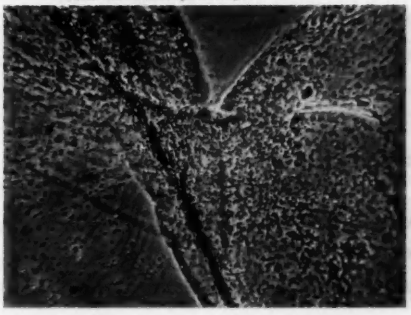


Fig. 5. Digestibility of 20 V.R.U./cc solution. A partial degeneration of the fibers and numberless pigment granules of ciliary body in the base (×400).

Fig. 6. Digestibility of 5 V.R.U./cc solution. The membranous structure of the surface is not clearly visible and numberless pigment granules are seen on this (×400).



the effect of the hyaluronidase in the vitro were similar to that in its intra vitrear injection in the vivo. Thus the author confirmed that the vitreous is made of fibrous structure and hyaluronic acid.

## Summary

1. The effects of hyaluronidase on the structure of the vitreous of dogs were studied under a phase-contrast microscope.

2. When hyaluronidase of high concentration (30-100V.R.U.) was injected into the vitreous, a partial degenerative change of the fibrous structure in both regions was observed.

3. When hyaluronidase of low concentration (5-20V.R.U.) was injected into the vitreous, a slight degenerative change on the surface structure of the vitreous was observed.

4. Subconjunctival hyaluronidase injection resulted in no change on the structures of the vitreous in case of low concentration, while it resulted in a slight degenerative change on the surface structure in case of high concentration (2,000-5,000V.R.U.).

5. A strong digestibility of hyaluronidase was observed on the surface structure in vitro.

(Published in Acta Ophthalm. Jap. 61: 1472, 1957 in Japanese)

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# STUDIES ON COLOR VISION
# REPORT I. THE SATURATION DISCRIMINATION

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In studies on color vision, three attributes, brightness, hue and saturation always become the principal subjects of discussion. It is a significant problem to do research on each attribute of color vision defect, but it is a more significant problem to do research on the correlation. Especially, the correlation betweem the due discrimination and the

saturation discrimination is an interesting problem for us. The definition of saturation which has been adopted by the Colorimetric Committee of the Optical Society of America is the following: "Saturation is that attribute of all colors possessing a hue, which determines their degree of difference from a grey of the same brilliance".

Naturally saturation is a subjective term. There is no color of 100 per cent saturation. As it is difficult to explain saturation itself objectively, conveniently the measure of saturation used is the least perceptible colorimetric purity. Least perceptible colorimetric purity may be defined as the ratio of the luminosity of the dominant hue to the luminosity of a mixture of white plus hue, where the mixture is judged to be just noticeably different from white or grey in hue, i.e. $p = \frac{L\lambda}{Lw+L\lambda}$. Saturation itself is not equal to the least perceptible colorimetric purity and is also not always parallel to the least perceptible colorimetric purity, but we can roughly derive from it the saturation discrimination.

## Apparatus

Fig. 1. General view of Hioki's anomaloscope being modified.

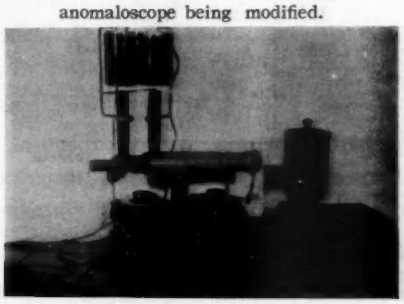
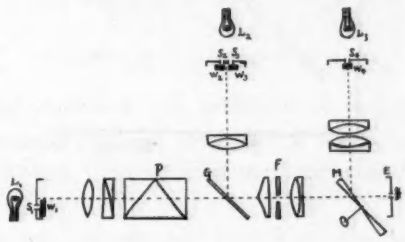


Fig. 2. Plan view of optical system of Hioki's anomaloscope being modified.



Hioki's anomaloscope was modified by the writer as in Figs. 1 and 2. Going into details, two of the three slits of the anomaloscope were not used, but only one slit $S_1$ was used. This anomaloscope was modified as to the part from the prism P to the observer's eye E as follows:

After the light from the slit $S_1$ passed through the prism P, the transparent glass G, the lenses and bi-prism, it focused on E. To measure the brightness, the sector of mirror M was set between the bi-prism and E. Moreover, white light from the upper light source $L_2$, passing through the pin-holes $S_2$ and $S_3$ 0.5 mm. in diameter, was projected on G; and so the smoked wedges $W_2$ and $W_3$ set under $S_2$ and $S_3$ changed the brightness by their movement. On the other hand, white light from the light source $L_3$ coming through the pin-hole $S_4$ 0.5 mm. in diameter fell on M. The smoked wedge $W_4$ was set under $S_4$ to change brightness of the white light from $L_3$.

The test area was divided vertically into two semicircular areas. White light from $L_2$ was put into the total area, and moreover the monochromatic light from $L_1$ was added to only one-half of the test area. When the lamp $L_2$ was operated at 106 volts, the

combination of the lamp and the filter S-T (Matsuda), yielded an energy distribution of 4800°K approximately.

The color temperature of the light source $L_1$ being operated at 98 volts was 2848°K. These color temperatures were always kept constant by the voltage stabilizer. The energy distributions of the light source $L_1$ and $L_2$ were as A and B curves in Fig. 3.

Fig. 3. The energy distribution of the light sources $L_1$ and $L_2$.

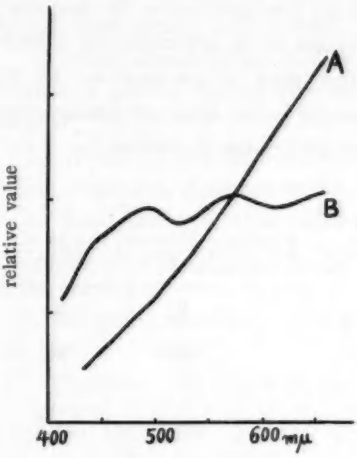
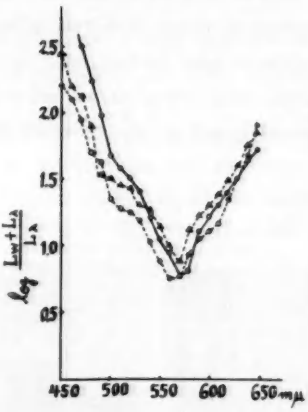



Fig. 4. Saturation discrimination curves for three normal observers.




## Method

The observer's eye was always adapted to a constant intensity of illumination of about 200 lux by which the screen was illuminated; and the test area was looked at while being illuminated at a constant brightness of about 0.001cd/m² (a retinal illumination about 1 Troland), under such condition as subtended 2° in a visual angle. First, the equal quantity of white light was put into the right and left half of the test area. Continuously, the hue was added to the right half step by step until the observers could say definitely that both of the sides differed in hue under the equal condition of brightness. For one hue, this procedure was repeated five times and was taken at 10 mμ. intervals between 650 mμ. and 450 mμ.. The threshold was taken as the mean value of the lowest proportion of hue always judged to be different from white.

## Results and Comments

As to the distribution curve of saturation thresholds for the nomal observer, the minimum value lies in the neighbourhood of 570 mμ. as in Fig. 4, and three normal observers show similar curves. The value in the blue region is bigger than in the red region. Therefore, it is supposed that the saturation discrimination in the blue region may be better than in the red region. In Figs. 5 and 6 are given the results of nine with

varying degrees of color vision defect, and the neutral points (the observer A. at 493 mμ.; E. at 503 mμ.; F. at 506 mμ.) are marked with the vertical dotted lines in Figs. 5 and 6.

Fig. 5. Saturation discrimination curves for one protanopic, three protanomalous observers and for the normal trichromat.

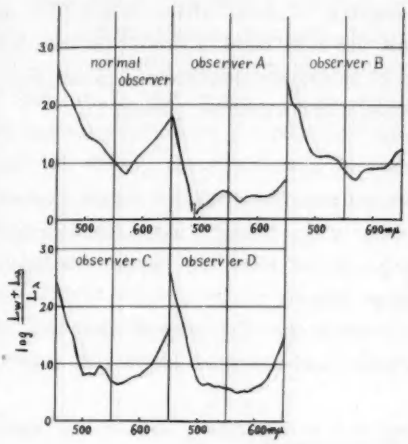
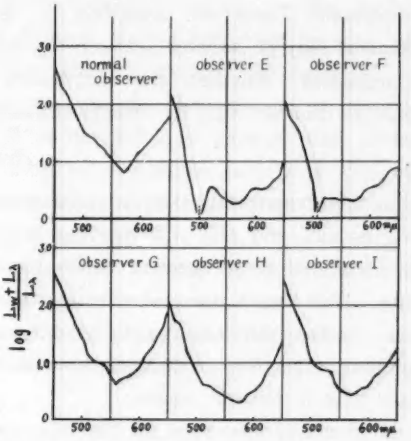



Fig. 6. Saturation discrimination curves for two deuteranopic, three deuteranomalous observers and for the normal trichromat.




In the first place, examining the color blind observers, the protanope A and the deuteranope E have the marked one minimum respectively at their neutral points, and have another minimum in the neighbourhood of 570 mμ., but the latter minimum only for the deuteranope F is not so marked.

Generally, the curves for the color blind observers are relatively smooth on the long wave-length side of the neutral points. The results of all the saturation thresholds for three color blind observers show low values which can not be measured at the neutral points, which are similar to those of Chapanis, Hecht and Shlaer. But the result that the value in the blue region is bigger than in the red region does not agree with that of Hecht and Shlaer, but agrees with that of Chapanis. On the other hand, the curves tending to rise between 600 mμ. and 650 mμ. differ from the smooth curves obtained by Chapanis, Hecht and Shlaer.

Secondly, examining the color anomalous, in three protanomalous observers, the curves of B and C reach their two minima in the neighbourhood of 500mμ. and 560mμ.. The observer D has relatively a smooth curve between 500 mμ. and 600 mμ., but his curve also appears to reach his minima at nearly the same positions. The curves for the deuteranomalous G,H and I reach their minima at nearly the same position as the normal observer. Only the curve of the observer I is somewhat smooth between 500 mμ. and 530 mμ.. In this point it differs from that of the other two observers. For the deuteranomalous, Nelson obtained, in connection with the hue discrimination curve, the smooth curve between green and red. The minimum of the curve that was obtained by Wright and Pitt lay rather in the shorter wave-length

side than the normal. In the color anomalous, the result that the curve rises more remarkably than that of the color blind observer between 600 mμ. and 650 mμ. differs from that of Chapanis, Nelson, Wright and Pitt. It is guessed that the saturation discrimination for the color anomalous generally becomes better in this range. As mentioned above, the color anomalous show various types of curves between the color blind observer and the normal. Therefore, according to varying degrees of color vision defect, the color anomalous may be situated between the normal and the color blind in view of the saturation discrimination. Further, the distribution curve of saturation thresholds does not always appear to coincide with the results obtained by Nagel's anomaloscope (Model 1).

## Summary

In this experiment, Hioki's modified anomaloscope was observed by twelve observes, three normal and nine with varying degrees of color vision defect. Saturation thresholds were measured at 21 spectral points as the reciprocal of least perceptible colorimetric purity. The results obtained may be summarized as follows:

1. In the distribution curve of saturation thresholds for the normal observers, the minimum value lies in the neighbourhood of 570 mμ. and the value in the blue region is bigger than in the red region.
2. Spectral saturation for the color blind observers is remarkably low at the neutral point.
3. The anomalous observers show low saturation in two regions, one in the neighbourhood of 570 mμ. and the other in the neutral point of color blindness, or in one region in the neighbourhood of 570 mμ..
4. It does not always coincide with the results obtained by Nagel's anomaloscope.
5. In view of the saturation discrimination, the color anomalous may be situated between the normal and the color blind.

(Published in Acta Ophth. Jap. 61: 1655, 1957 in Japanese)

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# STUDIES ON THE DEPTH OF FOCUS OF THE EYE

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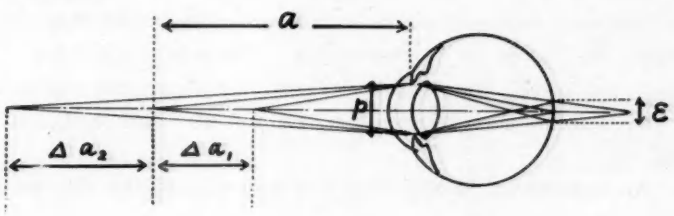
According to von Bahr's definition, the depth of focus of the eye is the difference between the maximum distances, expressed in dioptres, of 2 objects from the eye, which are perceived with maximum visual acuity. Theoretically speaking, the depth of focus of the eye is the range, within which man can see as distinctly as at the point conjugate optically with the receptor cell, and it is devided into anterior and posterior depth of focus. Some animals, for instance a bat or an octopus, have long rods, along which an image is thrown so as to be perceived equally, and therefore they have a wide depth of focus.

Let us suppose in Fig. 1, that "a" is the distance of a point object from the eye, "$\varepsilon$" is the diameter of the retinal image to be perceived as a point, "D" is the refractive power of the eye, and "$\Delta a_1$" and "$\Delta a_2$" are the anterior and the posterior depth of focus (in metres) respectively. Then in paraxial rays following equations are obtained.

Fig. 1.




$\Delta a_1$: the anterior depth of focus
$\Delta a_2$: the posterior depth of focus

$$\frac{1}{a-\Delta a_1} - \frac{1}{a} = \frac{\varepsilon}{p}\left(D - \frac{1}{a}\right) \cdots\cdots\cdots\cdots (1)$$

$$\frac{1}{a+\Delta a_1} - \frac{1}{a} = -\frac{\varepsilon}{p}\left(D - \frac{1}{a}\right) \cdots\cdots\cdots\cdots (2)$$

The left member in (1) or (2) indicates the anterior or the posterior depth of focus expressed in dioptres, and their absolute values are equal.

Many authors computed the values of the depth of focus from the above mentioned equations or the analogical one, but their values range from 0.07 to 0.3 D with an entrance pupil of 2 mm.. That was due to the difference of presumed values of $\varepsilon$. The eye differs from a camera, because above mentioned $\varepsilon$ of the eye is influenced not only by spherical and chromatic aberration as well as diffraction, but also by complex physiological factors. Therefore the computed values of the depth of focus of the eye do not hold true, as they are.

Ogle, Burian and Schubert, Miles, Hasegawa, von Bahr and others made experimental

measurements of the depth of focus, which are important for deciding the criterion of subjective estimations of visual acuity, refraction and accommodation, because the depth of focus relates to the threshold or accuracy of such estimations. I also made the experimental measurements of the depth of focus, and obtained some approach towards the solution of that problem.

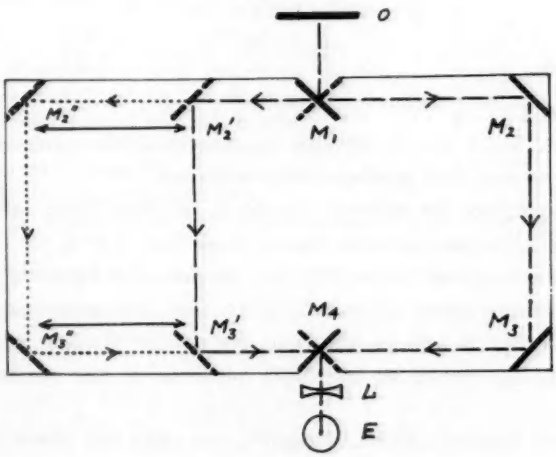
## Experiment

*Equipment.* In a mirror system, as Fig. 2 shows, the rays from the upper or the under half of the test object O passed through the right or the left optical path respectively, and coincided at mirror $M_4$ again. Therefore a subject E, whose chin and forehead were fixed, could see the image of O, which was reflected in $M_4$, through a round hole having a diameter of 5 cm.. The right optical path $OM_1M_2M_3M_4E$ measured 125 cm., and the left mirrors $M_2'$ and $M_3'$ could be slid together to the position of $M_2''$ and $M_3''$, thus the left optical length could be varied from 100 to 150 cm.. At L a lens was put, if necessary, in order to make a change in the accommodation of the subject's eye.

In the present experiment, colorless and colored test objects were used, and both were regular squares having sides of 7 cm.. The colorless objects were pasted at the left half of 4 kinds of colorless cards respectively (Albedo: 2.4%, 17%, 46% or 63%) made by a Japanese Color Company; because the right halves were white (Albedo 98%), their contrasts in percents were: 98, 74, 53 or 36 respectively. The colored object had a left half of black, and a right half of red, blue, yellow or gray. In these test objects it played a role, whether the boundary line between the left and the right half was appreciated distinctly or not.

*Procedure.* An incandescent electric lamp was used to illuminate the test objects, the

Fig. 2.



brightness of which was measured by means of Macbeth illuminometer, and amounted to 2, 18, 100, 300, 500, 1,000, or 9,600 rlx. A subject, whose pupil was dilated by using Neosynesin (Phenylephrin), saw through an artificial pupil having a diameter of 2, 3, 4, 5 or 6 mm.. The subject was the author (emmetropia, visus: 1,5). At first the left optical length of the equipment was made equal to the right one, and the subject gazed at the boundary line (somewhat above the center) of the test object. Then the left mirrors $M_2'M_3'$ were slid backward (towards left in Fig. 2), so as to lengthen the left optical path, and as soon as the subject became aware of a blur of the under half of the boundary line, he gave a signal. At that moment the left optical length was measured by reading a scale. Such procedure was repeated 6 times in succession, and the mean value was regarded as the limit of the posterior depth of focus. Then the mirrors $M_2'M_3'$ were slid forward (towards right), and the anterior depth of focus was measured in the same manner.

*Considerations of experimental procedure.* In order to measure the depth of focus,

Table 1. The Depth of Focus in Dioptres measured by using "White-black" Test Objects

| % Contrast | Brightness in rlx. | Pupil Diameter | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 mm | | 3 mm | | 4 mm | | 5 mm | | 6 mm | |
| | | ant. | post. | ant. | post. | ant. | post. | ant. | post. | ant. | post. |
| 98 | 2 | 0.037 | 0.044 | 0.027 | 0.026 | 0.025 | 0.021 | | | | |
| | 18 | .033 | .037 | .016 | .021 | .018 | .017 | | | | |
| | 100 | .027 | .026 | .016 | .019 | .012 | .013 | .010 | .011 | .011 | .011 |
| | 300 | .020 | .021 | .016 | .017 | .010 | .011 | | | | |
| | 500 | .023 | .020 | .012 | .015 | .009 | .010 | .007 | .008 | .010 | .008 |
| | 1000 | .022 | .019 | .011 | .014 | .008 | .009 | .008 | .008 | .009 | .010 |
| | 1600 | .018 | .017 | .008 | .013 | .009 | .008 | | | | |
| | 9600 | .016 | .010 | .007 | .008 | .006 | .007 | | | | |
| 74 | 2 | .042 | .045 | .034 | .035 | .026 | .022 | | | | |
| | 18 | .041 | .040 | .020 | .022 | .015 | .022 | | | | |
| | 100 | .031 | .030 | .020 | .021 | .014 | .016 | .013 | .015 | .013 | .014 |
| | 300 | .021 | .022 | .016 | .017 | .012 | .013 | | | | |
| | 500 | .023 | .023 | .016 | .016 | .014 | .014 | .013 | .012 | .012 | .013 |
| | 1000 | .022 | .021 | .015 | .015 | .012 | .012 | .012 | .013 | .013 | .013 |
| | 1600 | .019 | .019 | .015 | .013 | .010 | .010 | | | | |
| | 9600 | .015 | .014 | .012 | .011 | .010 | .009 | | | | |
| 53 | 2 | .042 | .048 | .037 | .032 | .028 | .026 | | | | |
| | 18 | .041 | .037 | .024 | .030 | .016 | .023 | | | | |
| | 100 | .032 | .021 | .024 | .024 | .016 | .017 | .016 | .014 | .013 | .013 |
| | 300 | .024 | .023 | .017 | .018 | .016 | .016 | | | | |
| | 500 | .026 | .024 | .020 | .019 | .014 | .014 | | | | |
| | 1000 | .024 | .023 | .019 | .018 | .013 | .014 | .014 | .014 | .016 | .014 |
| | 1600 | .023 | .022 | .022 | .019 | .019 | .011 | .015 | .014 | .015 | .016 |
| | 9600 | .019 | .014 | .014 | .014 | .009 | .009 | | | | |
| 36 | 2 | .052 | .050 | .037 | .037 | .034 | .029 | | | | |
| | 18 | .041 | .044 | .026 | .031 | .020 | .023 | | | | |
| | 100 | .033 | .035 | .023 | .025 | .020 | .022 | | | | |
| | 300 | .026 | .027 | .023 | .021 | .018 | .019 | | | | |
| | 500 | .027 | .027 | .023 | .022 | .020 | .019 | | | | |
| | 1000 | .028 | .026 | .023 | .021 | .019 | .020 | | | | |
| | 1600 | .028 | .025 | .023 | .023 | .019 | .020 | | | | |
| | 9600 | .029 | .027 | .023 | .021 | .014 | .016 | | | | |

Table 2. Influence of Accommodation on the Depth of Focus

| Brightness in rlx. | Accommodation in dioptres | Test objects | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | White black | | Gray black | | Red black | | Blue black | | Yellow black | |
| | | ant. | post. | ant. | post. | ant. | post. | ant. | post. | ant. | post. |
| 100 | 0.3 | 0.016 | 0.017 | 0.020 | 0.021 | 0.008 | 0.011 | | | 0.013 | 0.014 |
| | 0.8 | .016 | .019 | .017 | .018 | .009 | .011 | .007 | .009 | .013 | .014 |
| | 1.8 | .020 | .020 | .021 | .027 | .011 | .013 | .010 | .012 | .014 | .016 |
| | 2.8 | .021 | .024 | .025 | .028 | .013 | .017 | .011 | .016 | .014 | .015 |
| | 3.8 | .023 | .025 | .027 | .029 | .015 | .018 | .016 | .018 | .015 | .016 |
| | 4.8 | .025 | .029 | .031 | .036 | .019 | .023 | .018 | .023 | .016 | .017 |
| | 5.8 | .026 | .030 | .032 | .040 | .019 | .024 | .017 | .021 | .018 | .020 |
| 500 | 0.3 | .012 | .014 | .015 | .017 | .006 | .009 | | | .011 | .012 |
| | 0.8 | .012 | .015 | .013 | .012 | .006 | .009 | .005 | .008 | .010 | .011 |
| | 1.8 | .015 | .016 | .017 | .022 | .007 | .010 | .006 | .008 | .012 | .013 |
| | 2.8 | .018 | .021 | .020 | .024 | .009 | .013 | .008 | .015 | .012 | .013 |
| | 3.8 | .018 | .019 | .022 | .023 | .012 | .016 | .015 | .014 | .013 | .014 |
| | 4.8 | .018 | .025 | .025 | .030 | .017 | .022 | .016 | .022 | .014 | .015 |
| | 5.8 | .022 | .025 | .027 | .034 | .018 | .022 | .014 | .017 | .016 | .018 |
| 1000 | 0.3 | .011 | .012 | .014 | .015 | .005 | .006 | | | .010 | .010 |
| | 0.8 | .011 | .014 | .012 | .009 | .005 | .008 | .004 | .007 | .009 | .010 |
| | 1.8 | .013 | .014 | .015 | .019 | .006 | .008 | .005 | .007 | .011 | .011 |
| | 2.8 | .016 | .020 | .017 | .022 | .007 | .011 | .006 | .015 | .011 | .012 |
| | 2.8 | .016 | .017 | .020 | .020 | .010 | .015 | .014 | .013 | .012 | .013 |
| | 4.8 | .015 | .024 | .023 | .027 | .017 | .021 | .016 | .021 | .013 | .014 |
| | 5.8 | .021 | .023 | .025 | .031 | .018 | .021 | .013 | .015 | .015 | .017 |

while Hasegawa and Miles slid test objects backward or forward, von Bahr divided the optical path into the right and the left, in one of which a lens was interposed, so as to vary the vergens of the optical path. If the optical length is varied directly, as Hasegawa and Miles did, the visual angle subtended by a test object becomes smaller as the object goes away, therefore it becomes more difficult to discriminate it, and vice versa; thus the posterior depth of focus will measure too short, and the anterior one too long. On the other hand if a lens is interposed as von Bahr did, the image is enlarged by a convex lens and reduced by a concave lens, therefore the effects opposite to the former happen; that is, the posterior depth of focus will measure too long and the anterior too short. Moreover the vergens cannot be varied in succession.

Hasegawa used Landolt's ring as a fixed object and longitudinal parallel lines as a movable object, Miles' fixed object was a reduced Snellen's chart and the movable one was a light point, while von Bahr used 2 parallel lines, which seemed to be 3 lines through the von Bahr's equipment, because the one optical path made them an inverse image and the other made an erect one, and the one line of both images were overlapped. An observer gazed at the central line and signaled when he could narrowly discriminate 3 lines.

At first I used 2 parallel lines as test objects, but I noticed that by using them, larger values were obtained than the real one. The reason is, that because the threshold to discriminate 2 lines varies at each moment, we cannot use 2 parallel lines which extend a threshold visual angle constantly, and therefore a larger test object must be used than a threshold one.

Then a boundary line mentioned above was used as a test object, by using of which

it was not necessary to consider the change of the visual angle with the distance of objects. However, it required a great deal of skill, and fatigued the subject. On the basis of 18 measurements at the brightness of 1,000 rlx, the standard deviation was±6,5 mm., and the means of each 6 measurements, which were made at 3 days in succession, did not exceed the limits of±3,4 mm..

## Results

The measured values of the depth of focus were put instead of $\Delta a_1$ and $\Delta a_2$ in the equatinos (1) and (2), and 1.25 m instead of "a". Thus the depths of focus were obtained expressed in dioptres, which are shown in Table 1 and 2.

*Pupil and the depth of focus.* The effects of varying size of the pupil on the depth of focus are shown in Fig. 3 (at 100 rlx) and Fig. 4 (at 1,000 rlx), where the abscissae indicate a pupil diameter in mm., and the ordinates a depth of focus in dioptres (sum of the anterior and the posterior depth). Each 3 curves indicate the relation between the pupil diameter and the depth of focus by using the test objects, which have a % contrast of 98, 74 or 53 respectively.

In general, the depth of focus decreased in inverse proportion to the pupil diameter within the range from 2 to 4 mm., but if the pupil diameter exceeded 4 mm., the decrement became slower and even an increment happened. If the equations (1) and (2) held true, the relation discussed here should be expressed as a hyperbola, which were shown by broken lines in Fig. 3 and 4 where the pupil diameter is over 4 mm.. In reality the pupil influences aberation, diffraction and retinal illumination, which also affect the depth of focus. However,

Fig. 3. The relation of the depth of focus to a pupil diameter. (at the brightness of 100 rlx.)

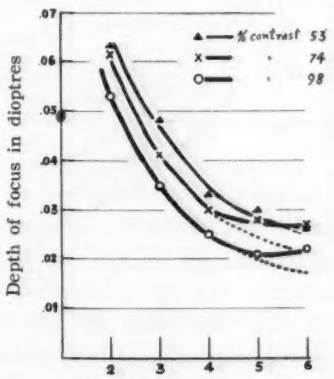
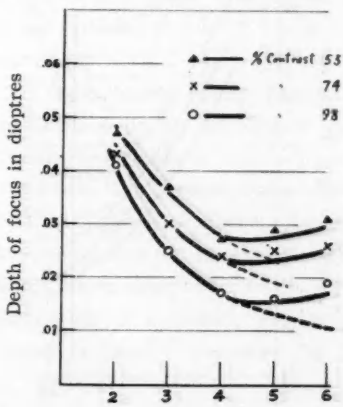



Fig. 4. The relation of the depth of focus to a pupil diameter. (at the brightness of 1,000 rlx.)



by the effect of retinal illumination only, the curves in Figs. 3 and 4 should deviate under the corresponding broken lines, and the diffraction becomes effectless if a pupil is dilated. Therefore it was considered that the deviation of the curves, obtained in the present experiment, from the theoretical hyperbola was due to the increase of aberration.

*Brightness and depth of focus.* The relation of the depth of focus to brightness is shown in Fig. 5 (with a pupil diameter of 2 mm.), Fig. 6 (3 mm.) and Fig, 7 (4 mm.), where the abscissae indicate the logarithm of brightness in rlx, and the ordinates a depth of focus. Each 4 curves indicate the results by using the objects, having a % contrast of 98, 74, 53 or 36 respectively. Here the decrease of the depth of focus with brightness were shown. Each 3 curves except the top are hyperbolas with easy slopes, within the range from 2 to 9,600 rlx, and the highest curves do so within the range from 2 to 500 rlx. These indicate the relationship:

$$d=\frac{k}{\log B}+k' \qquad \begin{pmatrix} d: \text{depth of focus} \\ B: \text{brightness} \\ k,\ k': \text{constant} \end{pmatrix}$$

Because $\delta$ in the equations (1) and (2) is in inverse proportion to visual acuity, it is considered that these datas in the present studies were the direct manifestation of the proportional relationship between visual acuity and the logarithm of brightness.

*Contrast and depth of focus.* As shown in Figs. 3~7, the worse the contrast, the more increased the depth of focus. But it was not reflected, that visual acuity was in proportion to the logarithm of % contrast, which was found by Hirasawa. It may depend on the

Fig. 5. The relation of the depth of focus to the brightness.(with a pupil diameter of 2 mm.)

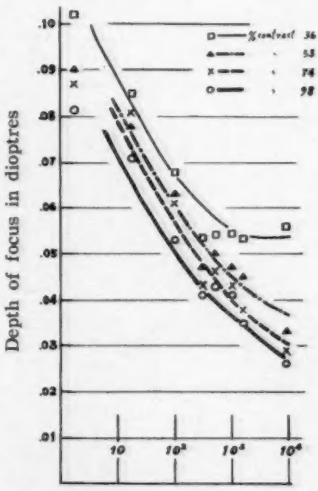



Fig. 6. The relation of the depth of focus to the brightness.(with a pupil diameter of 3 mm.)

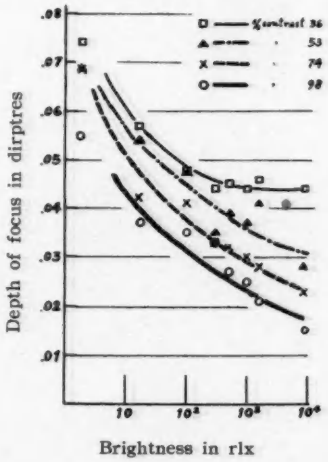


Fig. 7. The relation of the depth of focus to the brightness. (with a pupil diameter of 4 mm.)

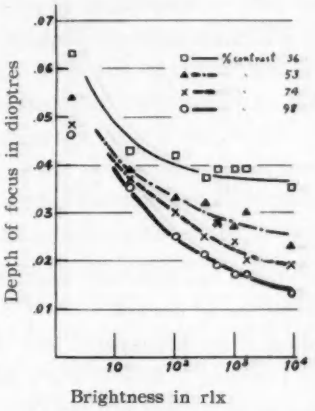



Fig. 8. The relation of the depth of focus to accommodation. (at the brightness of 100 rlx).

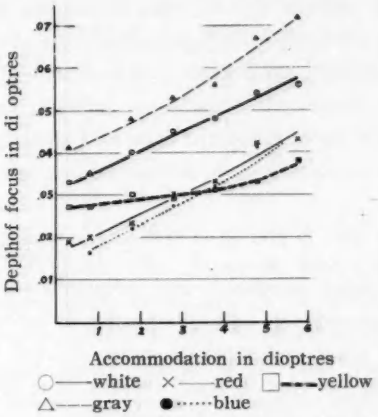
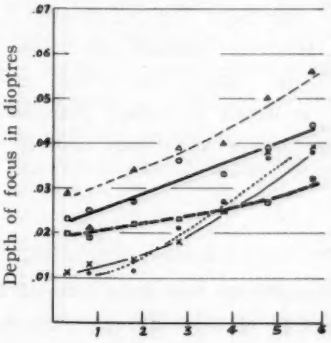



Fig. 9. The relation of the depth of focus to accommodation. (at the brightness of 1,00 rlx).




fact, that the experimental error could not be ignored, when the objects with low contrast were used.

*Accommodation and depth of focus.* In order to produce accommodation of a subject's eye, a +0.5, −1, −2, −3, −4 or −5 lens was inserted at L in Fig. 2.. Without a lens the accommodation amounted to 1/1.25=0.8 D, and with lenses it amounted to 0.3, 1.8, 2.8, 3.8, 4.8, or 5.8 D respectively.

The relations of the depth of focus to accommodation are shown in Fig. 8 (at the brightness of 100 rlx) and Fig. 9 (at 1,000 rlx), where the abscissae indicate the accommodation in dioptres and the ordinates the depth of focus. In all cases the depth of focus increased in proportion to accommodation, and the relationship $d=cA+c'$ (d: depth of focus, A: accommodation, c,c′: constant) was shown.

In the equations (1) and (2), although accommodation D increases, D−1/a remains constant, on the assumption that the principal point is fixed. Therefore the depth of focus in dioptres must be constant.

The movement of the principal point, accompanied with accommodation, must be too slight to explain the present results. It was considered, that the reason, why the depth

Table 3. Correlation between the Difference of Refraction, estimated by the subjective and the objective Methode, and the Visual Acuity with Cerrecting Lenses

| Difference in dioptres | Visual acuity | | | sum |
|---|---|---|---|---|
| | 0.1 to 0.4 | 0.5 to 0.9 | over 1.0 | |
| 0 | 62 | 108 | 416 | 586 |
| 1 | 43 | 83 | 186 | 312 |
| 2 | 34 | 19 | 39 | 92 |
| over 3 | 44 | 20 | 8 | 72 |
| sum | 183 | 230 | 649 | 1062 |

$\chi^2=170.34$ n=6
$P<0.001$ (significantly differenced)

of focus increased with accommodation, was to be found in spherical and chromatic aberration, as well as vibration of accommodation or fixation of the eye.

*Color and depth of focus.* In the present experiment monochromatic light was not used, so that I must refrain from drawing detailed inference. Figs. 8 and 9 show that the depth of focus decreased by using colored test objects (red : thin full line, blue : dotted line, yellow : thick broken line), compared with a white black object (thick full line) or a gray black one (thin broken line). The decrease of the depth of focus by using colorod objects was probably due to chromatic aberration, but such slight decrease, as in the present experiment, seemed to be unreasonable, considering the larger values of chromatic aberration, obtained by Polack (2 D), Wolf (2.4 D), von Bahr (1.5 D), Ivanoff (1.57 D), Wald and Griffin (3.25 D) or Hartridge (1.37 D). However, because the range of the spectrum with high luminosity is relative shallow, the datas in the present experiment may be reasonable, on the assumption that if 2 gradients of retinal illumination are compared, a gradient with slower slope should become aware as a blur, and in such a manner a blurred contour can be perceived.

## Discussion

Compared with the values of depth of focus, obtained by von Bahr (1～1.5 D with 2 mm. pupil diameter) or Miles (0.4 D at 40 cm., 0.26 D at 80 or 600 cm.), the values in the present studies or by Hasegawa (0.08 D at 1 m. with 3 mm. pupil diameter) were smaller. That was probably due to the facts, that, as mentioned above, with a test object of parallel lines used by von Bahr, the depth of focus would be estimated larger than the real one, and that the blur of a point, as used by Miles, would be more difficult to become aware of, than that of a line.

The awareness of a blur is influenced probably by the width and the gradient of contrast in a blurred zone, which will be projected on the retina. Calculating from the datas in the present studies, the blur of the boundary line became aware, when a blurred zone, with breadth subtending a visual angle of 12 seconds of arc, was formed on the

retina; that was smaller than the minimum visual angle to discriminate 2 lines, that is about 40 seconds.

A blur of contour is applied to the criterion, according to which the near point of accommodation is estimated. But such a value of distance of near point is smaller than the one obtained by using Staper's 2 line-target or the minimum separable in Scheiner's experiment. That may be understood by above described calculation. Duke-Elder mentioned in his Textbook of Ophthalmology, that it is a classic method to estimate by asking the subject about the blur of the target. But it is considered that such a method gives more accurate values of near point than to discriminate 2 lines, because the awareness of a blur is keener than the discrimination of 2 points or lines.

In the subjective estimation of refraction, the principle, that the refraction of the eye is the power of the weakest (or strongest) concave (or convex) lens to give the best visual acuity, poses a danger to give the weaker (or stronger) grade of myopia (or hyperopia). Therefore for the purpose to obtain the more accurate refraction, it may be better to adopt the power of the lens, which gives the most distinct vision instead of the best visual acuity. Table 3 shows the relation of the difference between the values of refraction, obtained by retinoscopy and the usual subjective method, to the visual acuity with correcting glasses. This significantly differed by the kai-square test. This result was probably due to the fact, that the greater the size of a test type, the more the adjacent types differed in size. Hereupon it is considered, that the new principle of the estimation of refraction, proposed here, would be most useful, when the corrected visual acuity is bad. Now it must be born in mind, of course, that the principle, to adopt "blur or distinct" as a criterion, is to be used for the purpose of the *accurate* estimation of refraction, for instance in cases of experimental studies on refraction, or the determination of the indication of Fukala's operation. For usual practical purposes, i.e. to prescribe glasses, however, it must not be applied as it is. The prescription of glasses is influenced by many other factors.

## Conclusion

1. The author measured the depth of focus of the eye by using a specially devised equipment with a mirror system. The obtained values were smaller than those by other authors; that was probably due to the test objects used.

2. The depth of focus decreased with a pupil diameter, and deviated from the expected relationship in paraxial rays.

3. It decreased with the brightness of test objects. That was considered a manifestation of the relationship between visual acuity and brightness.

4. It increased with accommodation, and with colored test objects its values became smaller.

5. The boundary line was perceived as blurred, when the retinal image, expected geometric-optically, had a width subtending a visual angle of 12".

6. The worse the visual acuity with correcting glasses, the larger the difference

between the refraction estimated by subjective and objective methods.

7. For the purpose to estimate refraction exactly, the power of the strongest (convex) or the weakest (concave) lens, which gives the most distinct vision instead of the best visual acuity, is to be adopted.

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## BIOMICROSCOPIC OBSERVATION OF THE FOLLICLES

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The ophthalmologist in daily practice examines the patient's conjunctiva with naked eyes and diagnoses it simply that, "this conjunctiva has a follicle". It was so in the past and again, at present. But by excising many conjunctival follicles which by naked eyes look exactly the same, but by examining them histologically, we were able to distinguish 2 kinds which were fundamentally different, that is: one a follicle and other a pseudofolicle. The former is a sort of an organoid and has a germinal center, and the latter, although by naked eyes it looks similar to the former type, is simply an expression of an inflammatory tissue change.

These both can also be differentiated biomicroscopically. Therefore, when we find a follicle-like appearance of the conjunctiva macroscopically, we may express it for a time with a term follicular body (FB), and then later decide correctly whether it is a follicle (F) or a pseudofollicle (PF) after examining them by means of biomicroscopical or histological survey.

Follicular body (FB) — follicle (F)
Follicular body (FB) — pseudofollicle (PF)

Generally, the PF appears as one of the symptoms of the acute inflammation in certain cases of an acute conjunctivitis, and as the acute conjunctivitis heals, usually it becomes gradually small and eventually disappears.

On the other hand, as a rule, the F develops into the chronic inflammation of the conjunctiva, and even after the inflammation subsides, it remains in the conjunctiva for a long time and when the chronic inflammation continues then it will show the development

which will be mentioned later.

Histologically the PF found in an acute conjunctivitis is a part of a simple diffuse inflammatory region with numerous cellular infiltration and where the PF was recognized macroscopically, there were a little more cells than its surroundings, but there were no fundamental difference in the inflammatory pictures, although we do recognize the proliferation of the reticular fibers. In other words, it only presents pictures of a sort of an acute exudative inflammation. When the inflammation does not heal and becomes chronic, it gradually changes from an acute exudative inflammation to a chronic productive inflammation.

In the case of the F, the lymphocytes and the reticular cells and also the cells which are differentiated from the latter, are localized and form a group which can be clearly distinguished from the inflammatory pictures of its surroundings, as its pictures being entirely different. And also in most cases the germinal center can be recognized and the reticular fiber of that section is very sparse. Therefore, F shows clearly a different findings from that of the PF.

## The Differential Diagnosis of PF and F by Means of Biomicroscope

When the FB which can be found by naked eyes is examined biomicroscopically, the PF and the F can be differentiated by the difference of the state of distribution of the blood vessels which run inside the follicular bodies.

As shown in figure 1, in the case of PF the loops of the blood vessels proceed from its base to the ceiling and rise to the surface by penetrating the stroma. The vascular loops inside the PF are not arranged orderly, and it is seldom to see anastomosis between themselves.

On the other hand, in the F as shown in figure 2, the blood vessels crawl up the surrounding wall just as if covering the stroma, and then will form the network of blood vessels by anastomosing flatly underneath the epithelium which makes the ceiling. This is just as Dr. Nataf has explained already in his literature.

*On the subject of PF.* Let us consider the cause of the FB which occurs quite often in certain acute conjunctivitis. Supposing that in the case of an acute exudation of the acute conjunctivitis, the thin colloid or a fluid component of the blood exudes from the blood vessels, then the FB does not appear. It is because this exudation diffuses evenly into the surrounding loose tissues in short time. On the other hand, when the cells and the plasma exude from the blood vessels, they show the tendency to accumulate around the small blood vessels because they have very little diffusibility for themselves, and the tissues that react also become very active around the small blood vessels. Usually the blood vessels of the normal tissues take a very low position inside the flat subconjunctival tissue, but when the inflammation occurs, the subconjunctival tissues swell up and the blood vessels immediately become thick. Then the length of the blood vessels becomes long and will look just like a tree with many branches spreading under the epithelial

layer. From each branch the exudation of the cells begins, and then when the strong tissue reaction occurs with the small branch as a focus, the small branch from one of the tree easily forms a group which by naked eyes can be recognized as one PF. Therefore as shown in figure 1 the PF can be built up.

The PF which was formed as a product of an acute exudative inflammation, will be called as an acute pseudofollicle ($PF_1$). There is no anastomosis between each vascular loop which is distributed in the $PF_1$; $PF_1$ itself takes the same fate with the acute inflammation, therefore as the acute inflammation heals, it will also disappear and return to normal condition.

But of course when the inflammation of the conjunctiva does not subside and becomes chronic, then the $PF_1$ also becomes chronic. In this case, when examining biomicroscopically, one can see the blood vessel picture become more complicated which is caused by the gradual anastomosis occuring between the vascular loops, the appearance of the new vascular loop, or the winding of the blood vessels inside the PF. Again, histologically the new blood vessels, the growth of the reticular fibers, the appearance and growth of the fibroblasts and the reticular cells can be recognized, but the germinal center cannot be seen. We call such PF, a chronic pseudofollicle ($PF_2$).

Therefore we can classify pseudofollicle (PF) into acut ($PF_1$) and chronic pseudofollicle ($PF_2$).

That is:

PF → $PF_1$
PF → $PF_2$

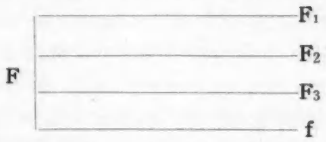
*On the subject of F.* It is possible to classify F as shown in figure 3, (F to f and their intermediate types), by examining biomicrospically the typical follicles more closely as pointed out in figure 2. $F_1$ is most young and its size is small, moreover the follicle is transparent and the number of the blood vessels distributed is few. Usually it is very difficult to distinguish the difference between the vein and the artery of these blood vessels. We express this sort of follicle as $F_1$. Histologically $F_1$ is made up of a group of lymphocytes which does not possess a germinal center, and the reticular fibers of the parenchyma are not as loose as the other follicles which will be mentioned later.

When the $F_1$ develops and the size becomes larger, there appears 1 or 2 of the central arterioles which penetrate the center of the stroma from the base of the follicle. At the beginning of the appearance of the central arterioles it is very hard to distinguish whether it is a vein or artery, but later will present cleary and external appearance of an artery. When the central arteriole reaches the ceiling of the follicle, it will anastomose with the veins which appears around it. The follicle which possesses the central arteriole of this sort is $F_2$. Histologically, a germinal center can be cleary observed but the reticular fibers are scarce in this part. The parenchyma which surrounds the germinal center is occupied mostly by lymphocytes, that is, a coat of lymphocytes.

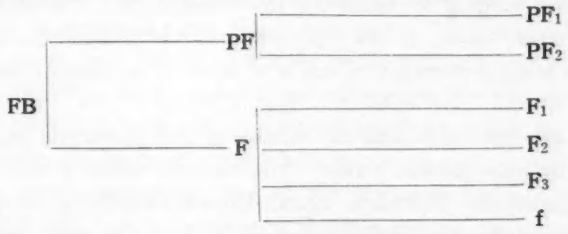
When $F_2$ develops further, it becomes $F_3$ of a bigger size. When $F_3$ is examined biomicroscopically, although the central arterioles disappear, the blood vessels that are distributed in the follicle are the same as in the case of $F_1$, and they encircle the parenchyma as if they embrace it. But in this case, as shown in the figure, the vein and the artery can be distinguished, and the follicles as a whole show somewhat a yellowish turbidity. Histologically, the parenchym of the follicle is placed by a reticular cells and its differentiated cells, and the reticular fibers are sparse, therefore a large germinal center is formed while the coat of the thin lymphocytes around it can be found.

The development of the F is as mentioned above, but the follicle does not always develop; on the other hand, it may retrograde. Some of $F_1$ and $F_2$ disappear without leaving any scars but most of $F_2$ and $F_3$ becomes cicatrized or either it will change into an atrophic follicle, that is, f. The f is a small follicle and very often it is covered by a conjunctival tissue, or it may at times be surrounded by a clear or a non-clear localized scar which makes it very hard to be discovered. The f remains for a long time in this state in the conjunctiva, but later it will disappear or will become scarified. Biomicroscopically the distribution of the blood vessel is similar to that of $F_1$. Only the winding of the blood vessels of f is stronger than that of $F_1$ and also they are not as transparent as that of $F_1$ because the parenchyma shows a little yellowish tinge. Histologically, a small atrophic germinal center and a small amount of lymphocytes which surrounds it can be recognized. Some times the coat of the lymphocytes is missing and only a naked follicle can be seen.

From the above reasons we can see that the follicle can be classified as follows:




Therefore, the follicular body of the conjunctiva which looks the same to our naked eyes can be differentially diagnosed biomicroscopically as following:




The Follicular Bodies seen in Trachoma

FB is from the olden times described as one of the most important symptoms of trachoma. $PF_1$ is seen first in any acute stage of trachoma which includes a subacute type A (Tr I (A)), a subacute type B (Tr I (B) ), acute trachoma (Tr I′) or the inocu-

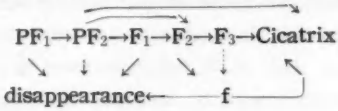
lated trachoma (Tr I′ inoculated). This in time becomes $PF_2$ as the inflammation of the conjunctiva becomes chronic, and then eventually the F will develop if the slow production of $PF_2$ exists for a long period of time. In other words F develops in the midst of $PF_2$ ground and in the beginning it appears as in the form of $F_1$ or $F_2$.

That is:

$$PF_1 \rightarrow PF_2 \rightarrow F_1 \rightarrow F_2$$

Of course in such cases, all the $PF_2$ does not become $F_1$ or $F_2$. The $F_1$ or the $F_2$ develops sporadically among the numerous $PF_2$. $F_1$ in the beginning may change into $F_2$ if the growth advances. Here if trachoma by chance heals, then $PF_2$, $F_1$ or $F_2$ disappears without leaving any scars, and again, $F_2$ may either become atrophic to form f, or $PF_2$ or $F_2$ may become scarified. On the other hand when the trachoma advances then the follicle develops, taking the course of $F_1 \rightarrow F_2 \rightarrow F_3$, furthermore the new $F_1$ or the $F_2$ will certainly grow in $PF_2$.

This entire development can be expressed with our signs as follows:




## Discussion

1) Primarily speaking, the development of the adenoid tissue of the subconjunctiva of the new born is very poor, and the lymphfollicle (F) cannot be found. At 3 months after birth the adenoid tissue develops all at once but the lymphfollicle cannot yet be seen. Not only that but even the germ of the lymphfollicle cannot be found. Therefore, if the lymphfollicle was discovered in the human conjunctiva then it is appropriate to consider it as a heteropoetic product. From this we can say that trachoma is clearly a follicle-, that is, a organoid-building disease and is an eye disease which should be classified as a specific inflammation in the place of general pathology. In biomicroscopic examination of the diseases not of trachoma, so far, we have not experienced in recognizing the growth of follicle which has a germinal center in the conjunctiva. Therefore, when a follicle possessing a germinal center in the conjunctiva can be recognized, then we can consider them entirely as being a trachoma or one of a signs of a residual stage of the past trachoma.

2) In the past we have been examining the diseases of the conjunctiva only by naked eyes and had been making many serious errors. The follicular bodies of the conjunctiva seen in the so-called conjunctivitis follicularis acuta, keratoconjunctivitis, atropin catarrh and conjunctivitis allergica acuta etc. were not of F but were of the acute pseudofollicles ($PF_1$).

We have been naming F and PF in these cases and in trachoma with the same terminology, therefore it caused an extreme confusion in understanding. With this interpretation, the conjunctival symptoms that do not develop F, namely, conjunctivitis follicularis acuta,

keratoconjunctivitis, atropin catarrh and acute allergic conjunctivitis etc., should hereafter be termed conjunctivitis pseudofollicularis, then we think that many of the ophthalmologist can avoid being confused with the knowledge of the so-called follicle.

3) We have mentioned chiefly on the findings of PF and F by biomicroscopic method in this literature but when making the differential table which is based on the findings of the F biomicroscopically and histologically, it will be as follows:

| | germinal center | central arteriole | lymphocytes or coating on lymphocytes surrounding the germinal center |
|---|---|---|---|
| $F_1$ | − | − | ±± |
| $F_2$ | ± | + | ± |
| $F_3$ | ±± | − | + |
| f | + | − | +~± |

Formerly, in the olden days the FB of the conjunctiva was classified macroscopically into follicle and granule. When classifying this old classification biomicroscopically, the so-called follicle corresponds to $PF_1$, $F_1$, $F_2$, and f and the so-called granules correspond to $PF_2$, the changing form of $F_2$ and $F_3$, and $F_3$. The differentiation between the two can be made by their form and the difference of their size, and also by their color and the grade of their transparency. But in general the follicle that possesses a great amount of reticular cells histologically shows the cloudy and the yellowish appearance macroscopically, and the ones that contains a great amount of lymphocytes looks clear and colorless. But such an old classification has hardly any meaning educationally as can be understood by the results of the biomicroscopical differentiation. The above classification is not giving very much of the fundamental difference.

4) Next we would like to show the actual anaylsis of the findings of the patients conjunctiva biomicroscopically according to our above interpretation.

Figure 4 A is a picture of the trachomatous conjunctiva which according to our classification belongs to $TrII_{ft}$. The follicular bodies of this conjunctiva when analyzing biomicroscopically is as shown in figure 4 B. That is, the FB that can be seen in great amount in the lower tarsal and fornix conjunctiva can be $PF_2$ or $F_2$, or else it may be F being formed inside the $PF_2$. In the upper tarsal conjunctiva the $PF_2$ and the $F_1$ can be recognized but as yet the $F_2$ possessing the central arterioles cannot be observed.

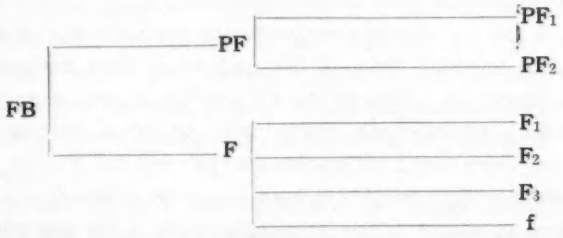
Generally, the development of papilla in the tarsal conjunctiva is the first symptom of trachomatous tarsal conjunctiva, and as the trachoma becomes worse individual papilla develops, and its size becomes enlarged. Furthermore, as the papilla keeps developing it will unite with each other and form $PF_2$, still further in the latter new $F_1$ and $F_2$ will start to grow. That is, it will take the course of, Development of papilla→Hypertrophy→fusion→$PF_2$→F, but at times the fusion of the papilla is not necessary and the development of $F_1$ can be recognized beneath the lined up in the base of many hypertrophic papilla.

Figure 5 A is also a picture of the macroscopic conjunctiva findings of the fornix and tarsal conjunctiva of the lower lid showing Folliculosis, and the biomicroscopic findings are as shown in figure 5 B.

These cases when treated with terramycin or achromycin show a qick disappearance of $PF_1$ and $PF_2$ parts but $F_2$ and $F_3$ show only a reduction of the size by the use of these drugs but does not readily disappear. Therefore even those trachoma which macroscopically look the same, if it is $PF_2$, then its conjunctiva will look clean externally in an early stage, but those that are $F_2$ or $F_3$ are very hard to get clean quickly. It is because the $F_2$ and $F_3$ is itself an organoid, and although the trachoma virus is killed by the use of drugs it is different from a simple inflammatory cells or the proliferation of the tissues, therefore it does not disappear immediately. Also, this itself may exist in the conjunctiva which has become normal after the other trachoma symptoms have died out just like the other lymphfollicles that can exist in the other parts of the body normally and may serve to be very convenient without giving any harm.

## Summary

In the past, we ophthalmologists were making many mistakes in diagnosing the findings of the conjunctiva due to the naked eyes examination only. Those follicular bodies (FB) of the conjunctiva that has the same external appearance macroscopically can be distinguished when examined histologically into lymphfollicle (F) which possesses a germinal center, and pseudofollicle (PF) which is simply an inflammatory change of the conjunctival tissue. This distinction can also be made biomicroscopically. Biomicroscopically the authors classify the FB into F and PF by the difference of the forms of the blood vessels which are distributed into it. Also, the F is classified into $F_1$, $F_2$, $F_3$ and f, and the PF into $PF_1$ and $PF_2$. This fact is found in perfect accordance with the result of the histologic research. Therefore, FB can be differentially diagnosed biomicroscopically and histologically as follows:




Formerly the FB that were seen in the so-called conjunctivitis follicularis acuta is $PF_1$. Therefore this should be changed to conjuctivitis pseudofollicularis acuta.

The course of the different forms of FB of the trachoma is as follows:

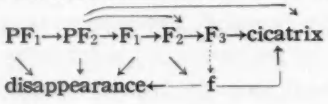
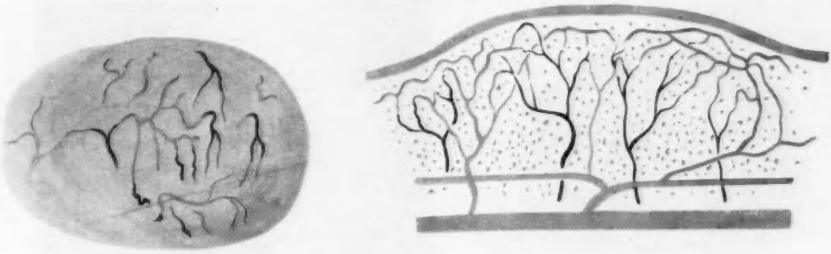
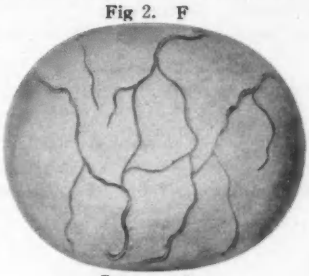


Fig 1. PF



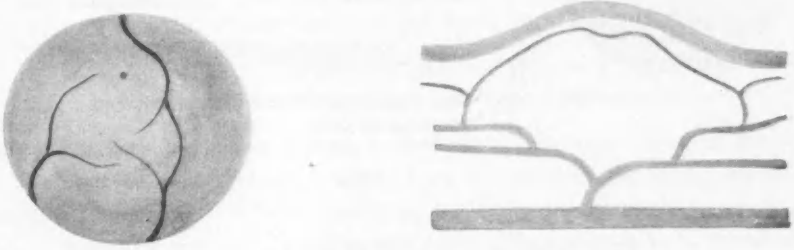
Black: artery Grey: vein
Dot: reticular cells and its differentiated cells.

Fig 2. F



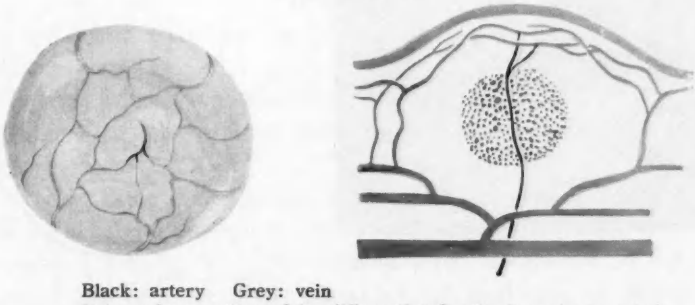
Grey: vein

Fig 3. (A) $F_1$



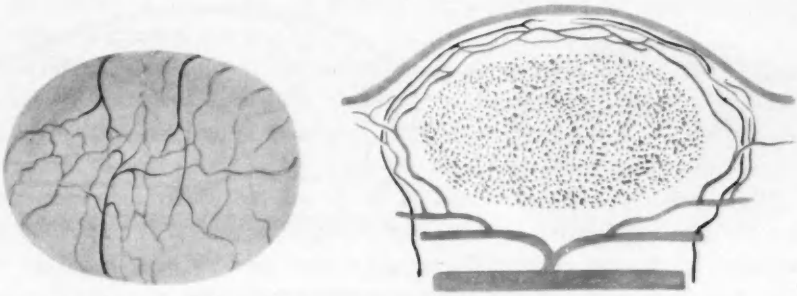
Grey: vein

Fig 3. (B) $F_2$



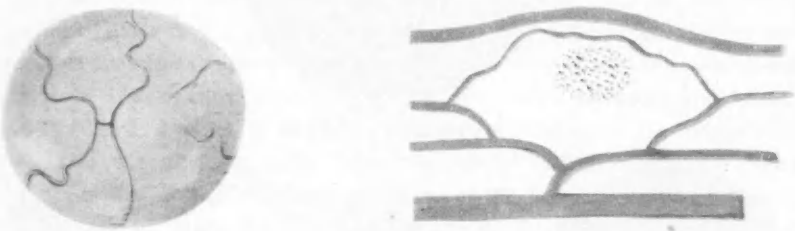
Black: artery Grey: vein
Dot: reticuar cells and its differentiated cells (germinal center).

Fig 3. (C) $F_3$



Blalkc: artery Grey: vein
Dot: reticular cells and its differentiated cells.

Fig 3. (D) F



Grey: vein Dot: reticular cells and its differentiated cells.

Fig. 4 (A)

(B)

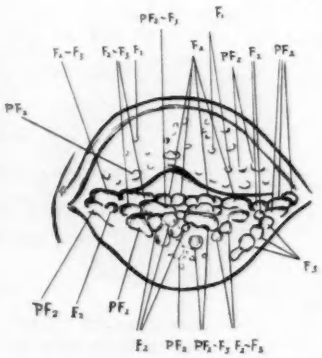
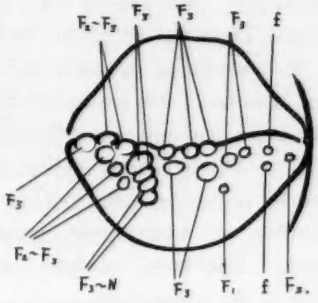


Fig. 5. (A) (B)

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## HISTOLOGY OF NORMAL AND TRACHOMATOUS CONJUNCTIVA AS REVEALED IN SECTION BY ELECTRON MICROSCOPY

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This is a basic study of our electron microscopy of trachoma virus in section (1,2). There has been no report in the literature on the electron microscopy of normal and trachomatous conjunctiva. Before undertaking the study on trachoma virus by electron microscopy, therefore, we had to examine the problem by ourselves as a control study.

The technique and method of the study is much the same as those described in other papers of the present authors (1,2). In this communication, some representative pictures obtained by this control study will be presented and a summarized description of the results will be made.

Figs. 1 and 2 are the representative findings of normal human conjunctiva. The material was obtained from normal adult and the pictures show the findings regarding upper retrotarsal fold.

Fig. 1 (×2,800) is the survey picture of the superficial layer of epithelium. The upper two thirds of the picture is occupied by the epithelial layer. The tunica propria is seen at the lower border of the picture. The epithelial layer is consisted of 3-5 layers of the epithelial cells. The cells are closely connected with each other by inter-cellular bridges

(ib). The inter-cellular space is very narrow.

As cell-organs, mitochondria (m), Golgi apparatus (G), and endoplasmic reticulum (er) can be seen. The Golgi apparatus by the nucleus should be differentiated from trachoma inclusion, as it shows an inclusion like appearance on the screen of the microscope. On the picture, however, it can easily be differentiated from the inclusion. In the subepithelium of this picture, a few number of fibroblasts (f) and some plasma cells (pl) can be seen.

Fig. 2 (× 18,000) shows epithelial cells at a higher magnification. A nucleus (n) surrounded by nucleus membrane (nm) is seen at the upper right corner. Various forms of mitochondria (m) with typical cristae, and various forms of endoplasmic reticulum (er) can be seen. The detail of inter-cellular bridge (ib) can also be seen. A lipid granule (lg) is seen at the lower right.

Fig. 3 (× 3,850) shows a survey of epithelial layer of trachomatous conjunctiva at the onset of the disease. The most characteristic changes are the invasion of leucocytes (W) into the epithelial layer and the formation of definite inter-cellular tunnels (t).

Two mucous cells (S) can be seen at the superficial layer of the epithelium. A number of leucocytes (W) are found in the inter-cellular space of epithelium. The connection of the cells by inter-cellular bridges has been broken and there is a network of tunnels (t) in the inter-cellular space. The tunnel may be formed by the invasion of leucocytes.

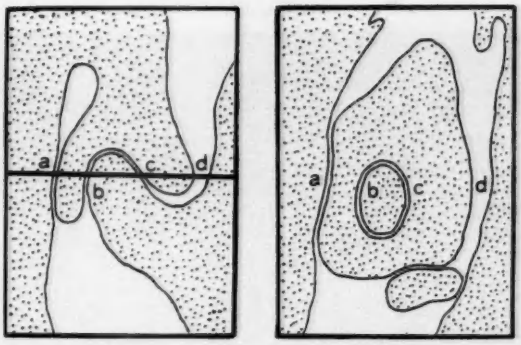
A blood capillary is seen at the lower border just beneath the epithelial layer. It is surrounded by a layer of endothelial cells (CE). It contains erythrocytes (R).

In trachomatous epithelium, mitochondria are often found in a group by the nucleus of epithelial cells (m). Such mitochondria should be differentiated from initial bodies of trachoma inclusions on the screen or at a low magnification.

It is obvious from this picture that the cells at the superficial layer of the epithelium are easily discharged from the underlying tissue owing to the looseness of the inter-cellular connection.

Leucocytes granules should also be differentiated from trachoma virus but the difference is not difficult.

Fig. 4 (×13,500) and fig. 5 (×16,200) are the epithelial cells of trachomatous conjunctiva at a deeper layer of epithelium. The mitochondria are swollen and edematous. There is an extraordinarily active development of inter-cellular bridges. Some of them look like pseudopodia of amoeba. The finding at the middle of fig. 5, is interesting. It looks like as if there is an elliptic corpuscle of about 300×350mμ., surrounded by double membranes. This "corpuscle" may be explained, however, by a cross section of a peninsula of a cell as schematically illustrated in the text figure. The apparent double membranes may not be an actual double membranes. Each of the membranes may belong to separate peninsula. When seen in vertical sections, such pseudo-double membranes may appear as the figures indicated by arrows in both of the pictures. Vertical and cross sections of endoplasmic reticulum (er), lipid granule (lg), and nucleus (n) of the cells are indicated by respective




Text-Figure. Schematical diagram of a portion indicated by a square in fig. 5. A: Postulated vertical sectitn of the portion. B: Schematical drawing of cross section as seen in fig. 5.

abbreviations.

Fig. 6 (×10,500) and fig. 7 (×3,300) illustrate the subepithelial change by trachoma. There are many epitheloid cells (ep), mast cells (ma), and plasma cells (pl). Remarkable endoplasmic reticulum (er) can be seen in a plasma cell.

Capillary endothelium (CE) and erythrocytes (R) in a capillary can be seen at the lower border of fig. 7. The epitheloid cells do not have such inter-cellular bridges as those of the epithelial cells. They are, therefore, not connected with each other. Mast cell granules should be differentiated from inclusions but the differentiation is not difficult.

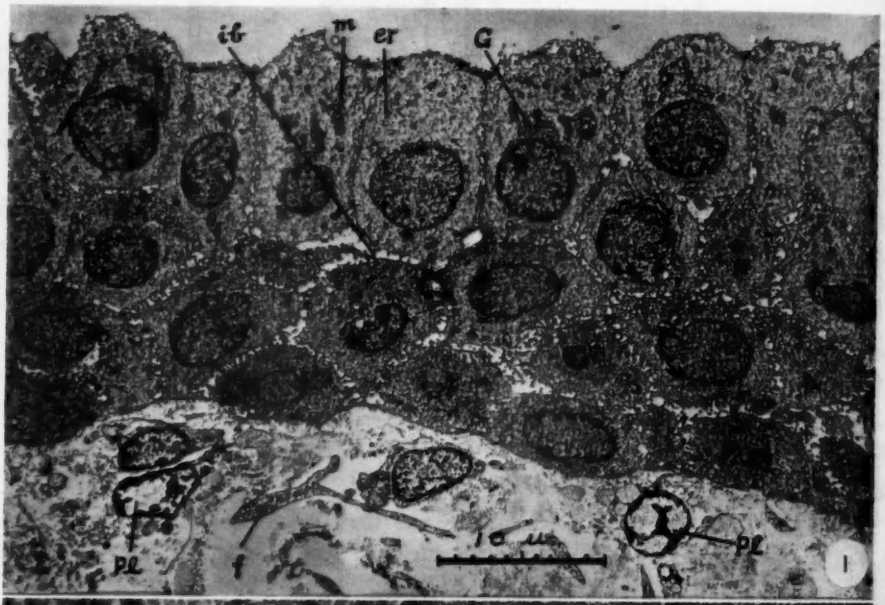
## Summary

On the screen of electron microscope, mast cell and leucocyte granules have certain resemblances with trachoma inclusions. They are, however, clearly differentiated each other by photography. Mitochondria are an important finding to be differentiated from initial bodies of trachoma virus in the picture. However, the fine structure of mitochondria enables us to differentiate the both.

On the screen, Golgi apparatus by the nucleus gives sometimes an impression as if it would be a cap-formed inclusion by the nucleus. This apparatus looks, however, entirely different from trachoma inclusions in the picture.

The most characteristic histological change by trachoma is found in the epithelial layer. As reported in separate papers (1,2), trachoma inclusions are only found in the epithelial layer and are never found in subepithelium. Within 24 hours after the onset of trachoma, the leucocytes invade the epithelial layer, destroying the inter-cellular bridge connection of the cells and forming " tunnels " in the inter-cellular space. The mitochondria of epithelial cells becomes swollen and the cristae are disturbed. Epithelial cells at the superficial layer

Fig. 1.



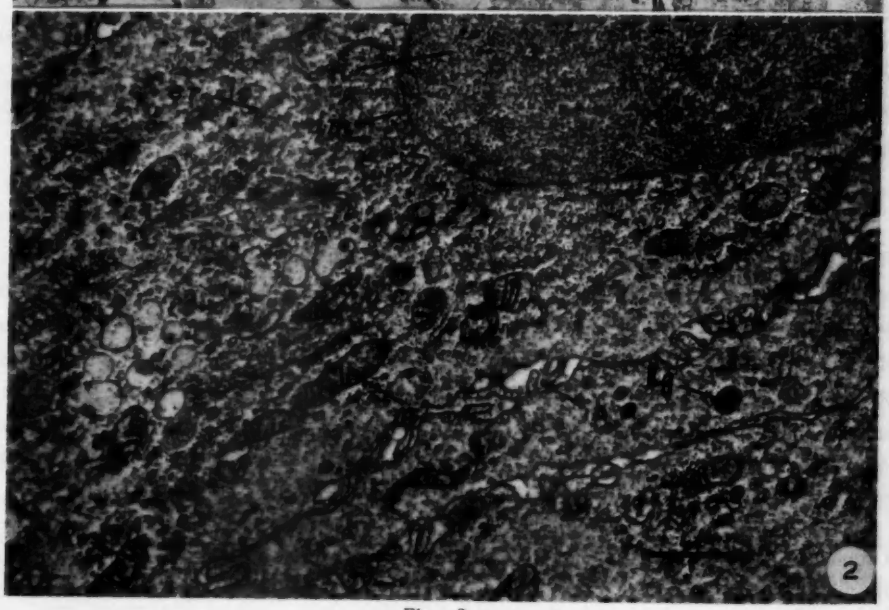




Fig. 2.

Fig. 3.

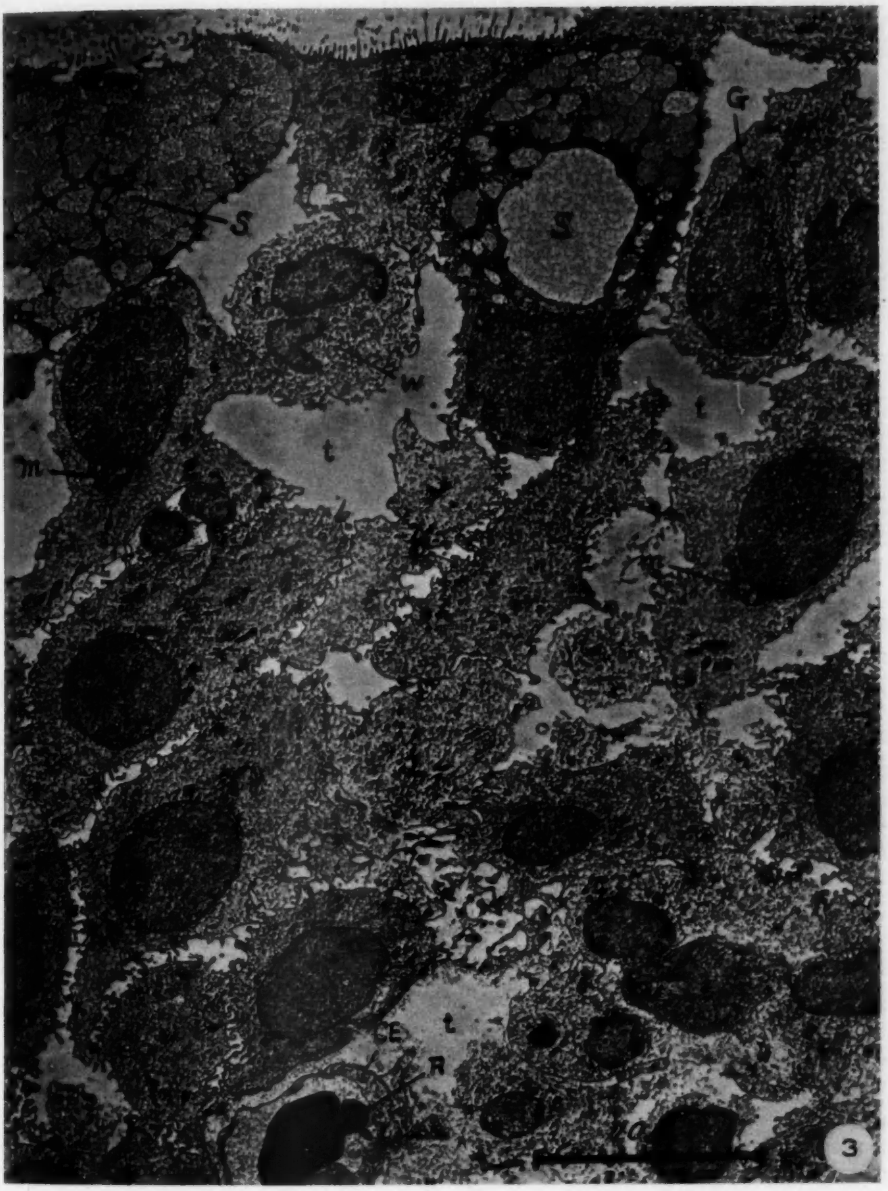


Fig. 4.

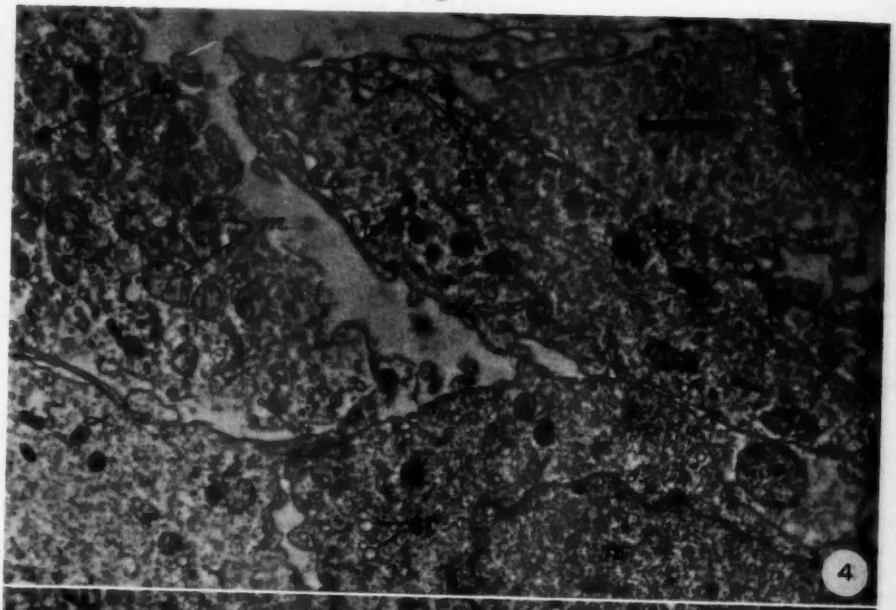
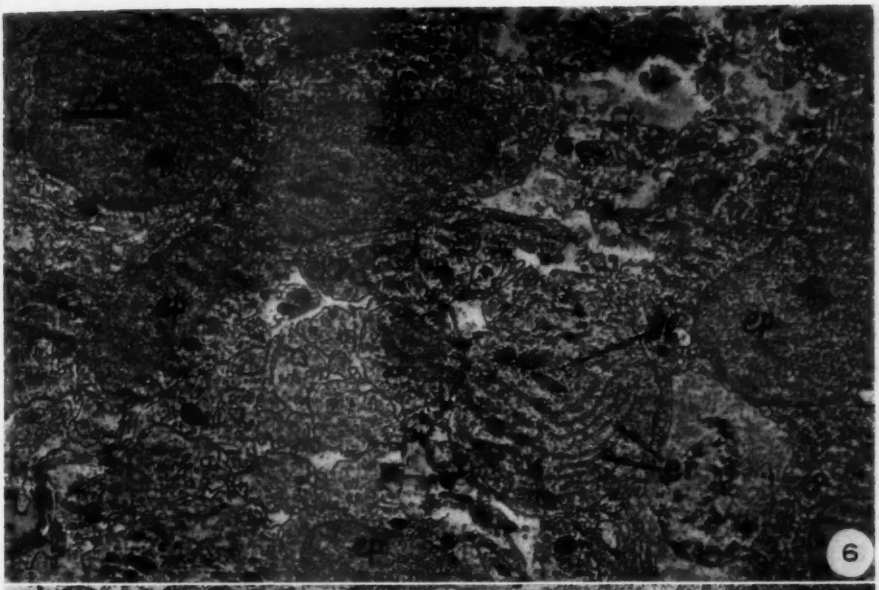


Fig. 5.

Fig. 6.



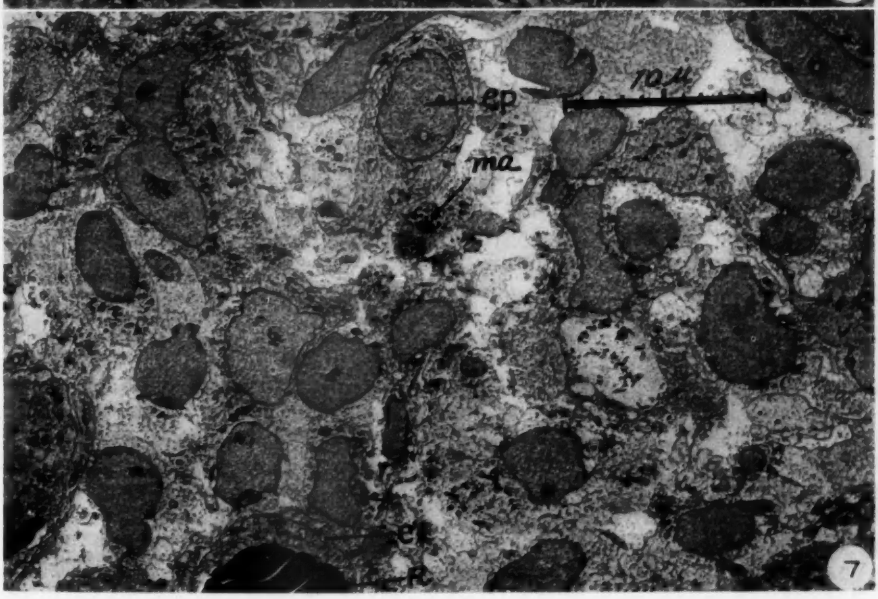




Fig. 7.

are apt to be discharged with mucous cells owing to the developed tunnels beneath the layer. Near the basal layer of the epithelium, the epithelial cells are apt to form processes just like pseudopodia of amoeba. The processes are complicated each other.

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# Editorial News

## Main Research Subjects of the Eye-Clinics

### Hokkaido University
1 Trachoma
2 Electronmicroscopic studies on the ocular tissues
3 Conjunctivitis and antibiotics

### Sapporo University of Medicine
1 Trachoma
2 Studies on the utility of the cerebral angiography in the neuro-ophthalmology

### Hirosaki University
1 Relation between Vitamine $B_2$ and metabolism of the ocular tissues
2 Malignant tumor of the eye
3 Electromyogram in the eye

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1 Eye-tuberculosis
2 Electrophysiological studies on some ocular diseases
3 Trachoma

### Tohoku University
1 Biochemical studies on the ocular tissues
2 Trachoma
3 Electronmicroscopic studies on the eye structure

### Fukushima Medical College
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2 Mechanism of accommodation, esp. presbyopia
3 Biomicroscopy of the eye

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1 Trachoma
2 Pigmentary degeneration of the retina
3 Lacrimal gland

### Chiba University
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2 Trachoma

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1 Corneal metabolism
2 Histological, physiological and electromyographic studies on the extrinsic eye muscles
3 Muco-cutaneo-ocular syndrome

### The Branch Hospital of the University of Tokyo
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2 Physico-chemical studies on the vitreous

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1 Slitlamp-microscopy
2 Uveal circulation
3 Glaucoma

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2 Hetero-keratoplasty

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2 Retinal blood pressure in the experimental arteriosclerosis and diabetes mellitus

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2 Vernal catarrh and diseases of conjunctiva and cornea
3 Allergy of the eye

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2 Viral infections in the eye

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1 Influences of nerve-shock upon the corneal pulse wave
2 Elasticity of the cornea
3 Electronmicroscopic studies on the cornea

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2 Trachoma
3 Hypertention

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2 Diseases of the sclera

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2 Surgery of the anterior chamber and cornea
3 Contact lens

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2 Electric tonometer
3 Depth of focus of the eye

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2 Gonioscopy and aqueous drainage channels
3 $\beta$-ray therapy

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2 Measurement of the blood pressure in the retina
3 Antibiotic therapy

### Shinshu University

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2 Visual purple

### Kanazawa University

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2 Visual processes in the retina (based on electrophysiology)

Nagoya University
1 Cytochemistry of the retina
2 Cone potential of the retina
3 Visual acuity

Institute of Environmental Medicine, Nagoya University
1 Visual perception of moving objects
2 Aetiology of refractive errors
3 Trachoma

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1 Relation of the fundus diseases to general conditions

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1 Syphilis of the eye
2 Allergy of the eye
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1 Actionpotential of the eye
2 The effect of the ultrasonic radiation on the eye
3 Studies on Na. and K. of the aqueous humor

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1 Trachoma
2 Squint
3 Biochemical studies on the optic nerve and tract

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1 Thiamine distribution of ocular tissues and its meaning in correlation with hormones
2 Ocular tissues under the phase-contrast microscope
3 Tonography

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2 E.R.G.
3 Histochemical studies on the retina

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2 Visual purple
3 In vitro culture of removed eye tissues

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2 Trachoma

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1 Pathogenesis and treatment of ocular diseases with special reference to stress

Nara Medical College
1 Biophysics of vision
2 Trachoma

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1 Glaucoma
2 Structure and functions of the retina

Kobe Medical College
1 Electromyographic studies of the extrinsic eye muscles

2 The relation of the disturbance of cerebrospinal fluid circulation and optic nerve diseases
3 Etiologic inquires into chronic retrobulbar neuritis, esp. on the arachnitis cisterna chiasmatis by defficiency and intoxication

### Okayama University
1 Glaucoma
2 Trachoma
3 Laparectomy of the eyeball

### Hiroshima University
1 Retinal detachment
2 Treatment of vitreous opacities

### Tottori University
1 Pigmentary degeneration of the retina
2 Glaucoma
3 Experimental cataract

### Yamaguchi Medical College
1 Ocular involvement caused by Co-poisoning
2 Trachoma
3 Viral infections of the eye

### Tokushima University
1 Ocular allergic reaction caused by lens components
2 Metabolism of polysaccharides in the ocular tissues
3 Experimental cataract

### Kyushu University
1 Neuro-ophthalmology
2 Squint
3 Trachoma

### Kurume University
1 Pathology of the eye diseases, esp. Sympathetic Ophthalmitis and Trachoma
2 Tissue culture of virus diseases of the eye
3 Electronmicroscopic studies on the ultra-sections of the eye

### Nagasaki University
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2 Experimental radiation cataract
3 Abnormal maternal environment upon fetal eye development

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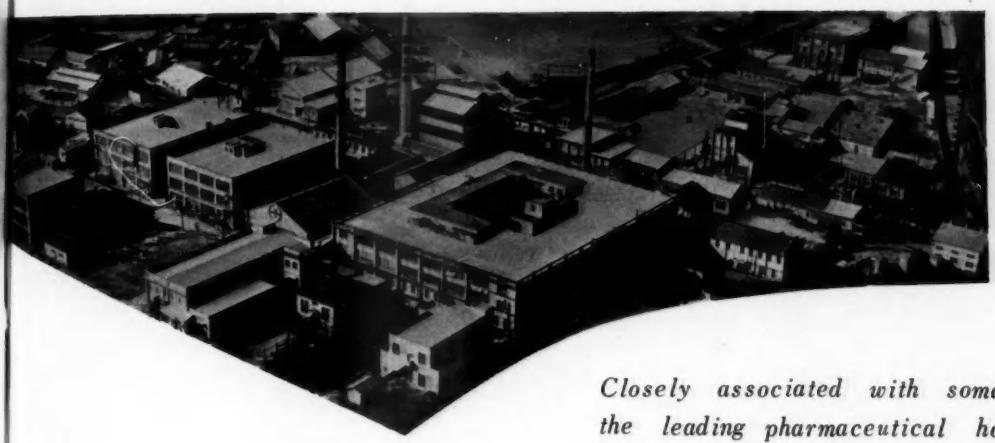

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